DISSERTATION ON

A STUDY OF HEAART RATE VARIABILITY AND LIVER FUNCTION TEST IN ALCOHOL DEPENDENCE SUBJECTS

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MD PHYSIOLOGY (BRANCH V)

DEPARTMENT OF PHYSIOLOGY

GOVERNMENT STANLEY MEDICAL COLLEGE
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CERTIFICATE

This is to certify that this dissertation entitled **"A STUDY OF HEART RATE VARIABILITY AND LIVER FUNCTION TEST IN ALCOHOL DEPENDENCE SUBJECTS"** by the Post Graduate **Dr. M.INDUMATHI** for **M.D. (PHYSIOLOGY), BRANCH – V** is a bonafide record of the research done by her in the Department of Physiology, Government Stanley Medical College hospital, Chennai in partial fulfilment of regulations of the Tamilnadu Dr MGR Medical University for the award of degree of MD (Physiology) Branch –V during the academic period 2015 – 2018.

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DECLARATION

I, Dr. M.INDUMATHI, solemnly declare that this dissertation entitled, **"A STUDY OF HEART RATE VARIABILITY AND LIVER FUNCTION TEST IN ALCOHOL DEPENDENCE SUBJECTS"** is a bonafide and genuine research work done by me in the Department of Physiology, Govt. Stanley Medical College and Hospital during 2015–2018 under the guidance and supervision of **Dr. VIJI DEVANAND, M.D.,** Professor and Head, Department of Physiology, Stanley Medical College, Chennai – 600 001.

The dissertation is submitted to the The Tamilnadu Dr. M.G.R. Medical University, towards partial fulfilment of the University regulations for the degree of M.D. (Physiology), Branch V, examination to be held in April 2018.

Place : Chennai – 600 001

Date : **(Dr. M. INDUMATHI)**

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CERTIFICATE-II

This is to certify that this dissertation work titled **"A STUDY OF HEART RATE VARIABILITY AND LIVER FUNCTION TEST IN ALCOHOL DEPENDENCE SUBJECTS"** of the candidate **Dr. M.INDUMATHI** with registration number **201515051** for the award of **M.D. PHYSIOLOGY** in the branch of **V**. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **1%** percentage of plagiarism in the dissertation.

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LIST OF ABBREVIATIONS

AD : Alcohol Dependence

ANS : Autonomic Nervous System

HRV : Heart Rate Variability

SBP : Systolic Blood Pressure

DBP : Diastolic Blood Pressure

BMI : Body Mass Index

HR : Heart Rate

LF : Low Frequency

HF : High Frequency

VLF : Very Low Frequency

TP : Total Power

ms^2 : millisecond square

n.u : Normalized units

SDNN : Standard Deviation of average N-N interval

RMSSD : Root Mean of the Sum of Squares of difference between adjacent NN intervals.

NN50 : N-N intervals differing by more than 50 milliseconds.

dL : deciliter

LFT : Liver Function Test

GGT : Gamma GlutamylTransferase

ALT : Alanine Amino transaminase

AST : Aspartate Amino Transferase

ALP : Alkaline Phosphatase

IU : International units

1. INTRODUCTION

Alcohol a psychoactive substance with dependence properties, causes a large number of diseases, social and economic burden in societies[1]. 16.0% of people above 15 years engage in heavy episodic drinking. 139 million DALYs (disability adjusted life years) were attributed to alcohol consumption in 2012. 3.3 million deaths in 2012 were reported to be due to alcoholism.[2]

A recent global study done by" The Paris-based Organization for Economic Cooperation and Development" (OECD) published a report stating that "Alcohol consumption in India is up by 55% between 1992 and 2012 and it is third in terms of increase in intake only to Russian Federation and Estonia. More worrying is the young are getting initiated into alcoholism earlier and are also indulging in binge and hazardous drinking."

" The World Health Organization has given the Sustainable Development Goals (SDGs) for 2030, which was adopted by the United Nations General Assembly in September 2015. Target 3.5 of SDGs is to strengthen the prevention and treatment of substance abuse, including narcotic drug abuse and harmful use of alcohol."[3]

Alcohol dependence subjects, classified based on International Classification of Diseases rev 10 (ICD-10): (World Health Organization's classification 1990) have behavioural and mental disorders, an increased prevalence of cardiovascular disease, attenuated bone health, cancer, physical problems.

Alcohol has a deleterious effect on a multitude of systems within the body, adversely influencing neural function, cardiovascular physiology, metabolism, thermoregulation and skeletal muscle myopathy.[4] The highest number of deaths are due to cardiovascular disease followed by injuries, liver disease/cirrhosis and cancers.[5]

ALCOHOL DEPENDENCE (AD) also known as alcohol dependence syndrome or alcoholism is a cluster of behavioral, cognitive and physiological phenomena which develops after repeated alcohol consumption.[6] Long term heavy alcohol consumption may lead to progressive, chronic cardiac dysfunction such as cardiomyopathy, heart beat rhythm irregularities (arrhythmias), hypertension, and stroke.[7]

Studies have shown a great significance between Autonomic Nervous System and Cardiovascular morbidity including sudden death and malignant arrhythmias. Heart rate variability (HRV) represents the hallmark of such markers.

Previous studies showed significant decrease in Heart Rate Variability in Alcohol Dependent Subjects compared to non-alcoholic subjects. This is due to cardiac autonomic neuropathy that decreases the cardio vascular performance leading to sudden death due to arrhythmias. Thus HRV can be used as a non invasive test for assessing the cardiac autonomic dysfunction".

The sympathetic activity which is responsible for "the fight or flight response" increases heart rate and blood pressure. Parasympathetic activity which is conveyed through the vagus nerve, is responsible for relaxation and restoration of heart rate, in which heart rate and blood pressure decreases.[8] The body works most effectively when there is high parasympathetic activity.

One way to assess the functional capacity of the parasympathetic nervous system is by measuring Heart rate variability.[9] Low basal HRV is unfavorable in that it characterizes autonomic rigidity.[10] Measurement of short term (5 min) heart rate variability has been advocated as an indicator of autonomic neuropathy.[11]

“ Heart rate variability refers to the complex beat-to-beat variation in heart rate produced by the interplay of sympathetic and parasympathetic (vagal) neural activity at the sinus node of the heart. Importantly, heart rate (HR) is under tonic inhibitory control via the vagus nerve.”[12] Heart rate variability has been shown to increase spontaneously with intervention and treatment.[13]

Alcohol is metabolized in the liver. It initiates a variety of metabolic responses that affects the final hepatotoxic response. High alcohol consumption for many years is likely to present with liver disease. Alcohol effects on the liver may range from reversible fatty change of the liver and hepatitis to irreversible cirrhosis. Alcoholic hepatitis may be acutely fatal in about 50% of the patients. It is also a precursor of alcoholic cirrhosis where the mortality rate increases to 70%.[14]

A number of laboratory tests are available to assist in the diagnosis of alcohol consumption and its related disorders. Several biochemical and haematological tests, such as GGT, AST, ALT are established markers of alcohol intake. Early assessment of their liver function using liver function tests will help in preventing hazardous alcohol induced liver complications.

Markers of excessive drinking are the liver enzymes namely Gamma Glutamyltransferase (GGT), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT).[15]

GGT is one of the established tests for excessive alcohol consumption.[16] About 5% - 20% of dependent drinkers with normal liver histology showed elevated GGT.[17,18]GGT levels are predictive of future morbidity and mortality.[19,20,21,22] GGT was used as a successful screening and interventional tool in the Malmo study and the Tromso study in Norway.[23,24]

Amino transferases similar to GGT, act as markers of alcohol consumption, and also as indicators of hepatic damage due to alcohol.[25] In otherwise healthy person, the most common cause of elevated ALT is Alcohol.[26]

Significant increase in both conjugated and unconjugated serum bilirubin levels was observed in alcoholic patients.[27] Albumin is a potential subject of formation of adduct by acetaldehyde, an alcohol metabolite. This can stimulate immunoglobulin formation, thus causing a rise in globulins.[28] And, also such changes may be related to ethanol induced oxidative stress which slows down the hepatic protein catabolism rate.[29]

GGT together with aminotransferases, albumin and bilirubin levels can be used as a broad indicator of presence and severity of hepatic impairment. Taking into considerations the above factors, the present study was done with the following objectives:

1. To assess the autonomic function in Alcohol dependent subjects and Non-alcoholic individuals.
2. To evaluate the Liver function parameters in Alcohol dependent subjects and Non-alcoholic individuals.

REVIEW OF LITERATURE

HISTORY :

The history of alcohol consumption traces from ancient times to the present. For thousands of years alcoholic drinks have been produced and consumed by Humans. They have played an important role in facilitating relaxation; promoting conviviality and social cohesion; religion; providing medicinal, antiseptic, and analgesic benefits; quenching thirst; increasing the pleasure of eating; also providing pharmacological pleasure; supplying nutrition and energy and enhancing the quality and pleasures of life.

In Europe, at first alcohol use was primarily medicinal, but it spread rapidly as a popular social beverage in the 1600s. The functions of alcohol drinks in our society has been controversial and debated . Still today, a conflict of views exists as to whether alcohol is an attractive elixir or a dangerous poison.[30]

In the early 1950s alcoholism was recognized as a disease by the World Health Organization (WHO). Jellinek described the “disease concept of alcoholism” and the subtypes of alcoholism. Edwards and Gross were the first to describe “ Alcohol Dependence” in 1976. The essential elements of the syndrome provisionally includes: “salience of drink-seeking behavior; narrowing of drinking repertoire; increased tolerance to alcohol; repeated

withdrawal symptoms; repeated relief/avoidance of the withdrawal symptoms by further drinking; subjective awareness of a compulsion to drink; reinstatement of the syndrome after abstinence". Physical, mental and social disabilities accumulate in the alcohol dependent subjects.[31]

Alcohol, its types & topographical distribution :

Beverages containing ethanol (C_2H_5OH) are fermented from a number of organic materials comprising carbohydrates, but in some parts of the world, these products are prepared from plants, fruits, berries, various grains, honey or milk. Such fermented beverages may contain up to 14% ethanol.

Alcohol – public health perspective :

Alcohol affects at the population level, rather than the individual level, which is the main concern. Drinking behavior of a person is likely to influence and be influenced by those around the person.[32]

Etiology :

The initiation of alcohol consumption largely depends on religious, social, cultural and personality characteristics, though genetic characteristics are also related to impulsitivity and risk taking behavior.

Pharmacology and Nutritional Impact of Ethanol :

Blood levels of ethanol are expressed as milligrams or grams per deciliter, with ingestion of one typical drink ~0.02 g/dL.

A standard drink is10-12g as seen in

340 mL of beer,

115 mL of nonfortified wine,

43 mL of whisky,

Additional components called Congeners are present in these beverages that affect the taste and contribute to adverse effects on the body. The congeners are methanol, butanol, acetaldehyde, histamine, tannins, iron, and lead.

Beer (3 - 7% ethanol) is the most widely commercialized fermented beverage, prepared from barley or other grains, also apple and other fruit ciders. Other fermented beverages common in particular cultures are Sorghum and Millet beers in Eastern and Southern Africa, palm wine toddy in western Africa and the Indian subcontinent. In Mexico pulque which is prepared from the maguey cactus, and rice wine in East Asia.[33]

PHYSIOLOGY/ ABSORPTION OF ALCOHOL

Alcohol is absorbed in small amounts from mucous membranes of the mouth and esophagus, in modest amounts from the stomach and large bowel, and the major site of absorption of alcohol is the proximal portion of the small

intestine. 2-10% of ethanol is excreted directly through the lungs, urine, or sweat, but the majority is metabolized to acetaldehyde, primarily in the liver.

Alcohol is devoid of nutrients and it can interfere with absorption of vitamins in the small intestine and it decreases their storage in the liver with effects onfolate, thiamine, pyridoxine and vitamin A. Knowledge about the deleterious effects of alcohol helps the clinicians to identify alcoholic subjects and provides

Alcohol Metabolism :

Liver is the site of alcohol metabolism. The metabolism of alcohol in the hepatocyte initiates a pathogenic process, which involves the production of protein – aldehyde adducts, immunologic activity, lipid peroxidation and cytokine release.

Cytokine production is believed to be responsible for the systemic manifestations of alcoholic hepatitis.

Cytosolic alcohol dehydrogenase and microsomal enzymes (primarily CYP2E1) metabolize alcohol to acetaldehyde. Acetaldehyde is further metabolized to acetyl –CoA by aldehyde dehydrogenase. This is then broken down to acetate, which is further converted to carbon dioxide and water or it enters the citric acid cycle to be converted to fatty acids. The latter is a major

reason for induction of fatty liver by alcohol, although acetaldehyde is the prime toxin.

Acetaldehyde causes most of the injury to liver cells, and also induces collagen synthesis leading to fibrosis and ultimately cirrhosis. Alcohol causes damage to intestinal epithelial cells, leading to lipopolysaccharide release, which can also damage liver cells. Other metabolic changes observed in alcoholic liver disease includes changes in methionine metabolism and oxidative stress. Genetic factors also play a role in alcoholic liver disease, and they are also important in determining persons with risk of developing liver disease in alcoholics.

Beneficial effects of alcohol

At low doses alcohol causes decreased rates of myocardial infarction, stroke, Gallstones, and Alzheimer's dementias. Alcohol especially ethanol has many uses in human life. They include non-beverage uses as a fuel and also as a solvent, and their beverage - related uses as described by Makela 1983, are as a medicine, religious sacrament, as a foodstuff, and as a thirst quencher. Alcohol has an anodyne property and is indeed an anaesthetic: distilled spirits were used as an anaesthetic agent in surgical practice before the mid-nineteenth century. Ethanol is a depressant and they affect mood and feelings.[32]

Adverse effects of alcohol

Acute :

Acute effects of alcohol are associated with the particular drinking event. Physical coordination, cognition, and attention are progressively impaired, resulting in an increased risk of accidents and injury. Graham et al.1998 has described that drinking above a threshold level can affect intention, judgment, and intoxication plays a causal role in violent behaviours and crime. Potentially fatal overdose of alcohol interrupts various autonomic bodily functions. It also acutely decreases neuronal activity. "Legal intoxication" requires a blood alcohol concentration of 80 – 100mg/dL, and death can occur between 300 – 400mg/dL.[32]

Chronic :

Alcohol dependence / Alcoholism contributes to damage of tissues which include liver, central nervous system, peripheral nervous system, skeletal and cardiac muscle. Ethyl alcohol damages the vasomotor and cardiac autonomic nerve fibers leading to autonomic imbalance, with neurovascular and cardiac dysfunction. The cardiac dysfunction results in reduced heart rate variability.[32]

Effect of alcohol on Cardiovascular system:

Alcohol causes decreased myocardial contractility and peripheral vasodilation, resulting in mild decrease in blood pressure and a compensatory

raised cardiac output. Alcohol is a striated muscle toxin , with a resultant deterioration in the heart muscle which manifests as beating irregularities and signs of heart failure – alcoholic cardiomyopathy. Thus , the leading cause for early deaths in alcoholics is cardiovascular disease.[33]

Chronic heavy drinking may cause cardiomyopathy, their symptoms range from unexplained arrhythmias to heart failure and hypocontractility of heart muscle. One third cases of cardiomyopathy are alcohol induced. Heart rate variability constitutes a composite measure of balance between the sympathetic and the parasympathetic tones. In alcoholic neuropathy, there is functional and structural damage to both sympathetic and parasympathetic nerve fibers. They both individually contribute to impaired cardiac function.[33]

In other autonomic dysfunction related psychiatric diseases like depression and anxiety, biofeedback targeting heart rate variability by visualizing heart rate deviations on a computer screen demonstrated improved cardiac function by increasing heart rate variability.[34] A recent study has demonstrated HRV biofeedback in a short term setting to be feasible to treat substance abuse patients.[35]

Effect of alcohol on the Liver

Alcoholism is one of the major reasons for liver disease in the western world and also in India. Gluconeogenesis is impaired, with a fall in the amount

of glucose production; lactate production increases; and decreased oxidation of fatty acids, with an increased fat accumulation in liver cells. On repeated alcohol exposure, the changes that occur in the liver include fatty accumulation, alcohol induced hepatitis, perivenular sclerosis and cirrhosis.[31]

Pathology of Alcoholic Liver Disease:

Alcohol is a direct hepatotoxin. The duration and quantity of alcohol intake is the pathogenesis behind chronic alcoholism. It comprises of 3 major lesions which are

1. Fatty liver – 90% binge and chronic drinkers are vulnerable,
2. Alcoholic hepatitis
3. Cirrhosis.

In alcohol dependence there is increased alcohol intake resulting in the accumulation of proteins and fats in the hepatic cells, producing a reversible swelling described as fatty liver. If not abstained at this stage , it leads to a stage of hepatitis, in which there is inflammation of the liver cells along with a subsequent increase in some liver function tests.

This stage progresses to a state where excessive amounts of hyaline and collagen are deposited near the blood vessels called as an early stage of cirrhosis. As the damage advances and scar tissue increases, the normal blood

flow through the liver becomes impaired, leading to dilated veins or varices causing ascites.[33]

The fat accumulation within the perivenular hepatocytes corresponds with the location of alcohol dehydrogenase, which is the major enzyme involved in the alcohol metabolism. Continuing alcohol intake contributes to fat accumulation in the entire hepatic lobule and distortion of the hepatocytes.

But studies have revealed normalization of fat content and hepatic architecture in the liver on cessation of drinking. Fatty liver and alcoholic hepatitis are potentially reversible on abstention, whereas cirrhosis is irreversible.[33]

MANAGEMENT

1. Alcohol abstinence.
2. Nutritional support
3. Pharmacological treatment
    (i) Disulfiram – most commonly used alcohol sensitizing drug, and the only one approved by the US Food and Drug Administration. It acts by inhibiting alcohol dehydrogenase enzyme, which converts acetaldehyde to acetate in the liver.
    (ii) Naltrexone – pure opioid antagonist which reduces craving and relapse rate.
    (iii) Acamprosate – increases abstinent days.

(iv) Selective Serotonin Reuptake Inhibitors.

(v) Topiramate – reduces craving.[31]

(vi) Ondansetron – a selective 5-HT3 receptor antagonist produces better results in early onset alcohol dependence.[36]

(vii) Prednisolone 32mg per oral daily for 4 weeks

(viii) Glucocorticoids – as it involves cytokine release and perpetuation of injury by immunologic process, glucocorticoids are used in alcoholic hepatitis.

(ix) Pentoxifylline – 400mg per oral tid for 4 weeks. Used as an alternative to glucocorticoids. Acts by inhibiting TNF (tumour necrosis factor).

4. Brief Interventions and Motivational Interviewing:

The brief intervention is effective in decreasing the alcohol use and its problems. It is instituted as two 15 minute sessions 1 month apart, along with a telephone follow – up reminder. Motivational interviewing uses the clinician's level of concern.

The families of alcoholics can be referred for counseling to self help groups like Al-Anon (the Alcoholic Anonymous) group for family members) and Alateen (for teenage children of alcoholics).[32]

5. Good social support.[31]

ALCOHOL DEPENDENCE

Definition of Alcohol dependence :

" Alcohol dependence is defined as alcohol-seeking behavior, despite its adverse effects; AD is considered more serious and advanced form of alcoholism."

" Alcohol dependence is defined in the Fourth Diagnostic and Statistical Manual (DSM-IV) of the American Psychiatric Association as repeated alcohol-related difficulties in three of seven areas of functioning that cluster together over any 12 month period" that includes the following criteria:

(i) tolerance

(ii) withdrawal

(iii) alcohol used in larger amounts/ longer period

(iv) presence of a persistent desire to take alcohol

(v) Too much time spent in activities necessary to obtain alcohol, use alcohol, or recover from its effects

(vi) loss of interest in social, occupational, or recreational activities

(vii) continued alcohol use despite its adverse effects.[5]

The clinical diagnosis of alcohol dependence ultimately depends on the documentation of a pattern of difficulties associated with the use of alcohol; and not on the quantity and frequency of alcohol consumption. Alcohol ingested, distributes throughout the body tissues rapidly and its abuse contributes to the damage of variety of tissues, which includes liver, the central and peripheral nervous systems, and cardiac and skeletal muscle.

The risk factors for alcohol related neuropathy are thiamine deficiency, malnutrition[37], direct toxicity of alcohol[38] and also a family history of alcoholism, but there is no clear evidence which plays a primary role in causing neuropathy. Chronic Ethanol ingestion reduces thiamine absorption in the intestine, thereby reducing the hepatic stores of thiamine. It also affects the phosphorylation of thiamine, which helps converting to its active form.

AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system (ANS) controls the activity of cardiac muscle, smooth muscle and certain glands. It maintains the internal homeostasis of cardiovascular, gastrointestinal, genitourinary, exocrine, thermoregulatory, and pupillary functions. The ANS is actually controlled by the brain, especially the hypothalamus and medulla oblongata which receive inputs from the limbic system and various regions of cortex. The Autonomic Nervous System is divided into

1. The Sympathetic nervous system – thoracolumbar division
2. The Parasympathetic nervous system – craniosacral division

The sympathetic effect is long lasting, whereas the parasympathetic stimulation is short lived. The sympathetic nervous division prepares one for emergency situations (fight or flight response), causing loss of energy. Whereas the parasympathetic nervous division regulates the activities that conserve and restore the body energy (rest and digest). The autonomic reflexes regulate the activities of cardiac muscles, smooth muscles and glands. The final common pathway from the central nervous system to the visceral targets are through the sympathetic and the parasympathetic neurons.[39]

AUTONOMIC CONTROL OF THE CARDIOVASCULAR SYSTEM

The autonomic nervous system plays an important role in overall cardiovascular homeostasis. The heart is an effector organ which receives opposing influences from the parasympathetic and sympathetic divisions of the ANS. Nor-epinephrine is released from the postganglionic sympathetic nerves. This activates the β_1 – adrenoreceptors present in the Sino-atrial node, Atrio-ventricular node, His –Purkinje conductive tissue, and also the atrial and ventricular contractile tissue.

Thus the stimulation of the sympathetic nerves causes increased heart rate (chronotropy), increased force of ventricular contraction (ionotropy) and

increased rate of conduction (dromotropy). Acetylcholine is the neurotransmitter released from the postganglionic parasympathetic (vagus) nerves that activates the Nicotinic receptors present in the sino-atrial node, atrio-ventricular node and the atrial muscles of the heart. Thus the stimulation of the vagus nerve causes reduction in the heart rate, atrial contractility and rate of transmission through the AV node.

The neural influences to the heart comes from several parts of the forebrain, brain stem, and insular cortex. A group of neurons located adjacent to the pia surface of the medulla called the rostral ventrolateral medulla (RVLM) or Vasomotor area is one of the major sources of excitatory input to the sympathetic nerves. The medulla is also a major site for origin of excitatory input to the cardiac vagal motor neurons in nucleus ambiguous that reduces the heart rate.

The intrinsic heart rate is about 100 -120 beats per minute. But in a healthy individual, the heart rate ranges between 60 and 90 per minute. This is due to the balance between the sympathetic nerves, which accelerate the heart rate and the parasympathetic nerves (vagus), which slow the heart rate.[39]

Autonomic functions are evaluated by invasive and noninvasive tests. The invasive tests require complex procedures and are used for the localization

of the site of lesion. The noninvasive tests can be performed readily and are used to confirm the diagnosis of autonomic neuropathy.

There are a list of non-invasive autonomic function tests for the assessment of Cardiovascular function. They include heart rate response to tilting, heart rate variation with respiration, heart rate and blood pressure response to standing, valsalva ratio, isometric exercise and cold pressor test.[40]

HEART RATE VARIABILITY

Definition :

"HRV , heart rate variability is the degree of fluctuation in the length of the intervals between heart beats."(Malik & Camm,1995)

"Heart rate variability refers to beat to beat variation in heart rate between two consecutive beats as oscillation between consecutive instantaneous heart rate. HRV is mirroring the regularity of heart beats: bigger regularity – lowers HRV (and vice versa). Regularity of heartbeats is derived from a quantity of numbers; equal to the time elapsed between successive heartbeats. They are named R – R intervals / N-N intervals and are measured in millisecond (ms).[41]

The physiological origin of HRV is the fluctuations in the activity of cardiovascular vasodilatory andvasoconstrictory centers in the brain. Factors like blood pressure oscillation which is baroreflex modulated; thermoregulation;

respiration; and circadian biorhythm influence the length of beat-to-beat intervals, namely R-R intervals / N-N intervals. Depressed / Reduced HRV means the heart rate is monotonously regular. It denotes lowered ability of the autonomic nervous system's regulatory function, and a lowered ability to maintain the homeostasis, deficiency in coping up with the external and internal stressors and in resisting disease or recovery in appropriate time.[41]

The variation of heart rate was analyzed using time domain and frequency domain methods for a short term period of 5 minutes to provide the degree of balance and activity of autonomic nervous system". HRV can be assessed in two ways

1. Time domain analysis and
2. Frequency domain analysis[41]

TIME DOMAIN ANALYSIS (5 MINUTES)

" This is calculated based on statistical operations on 'normal-to-normal' (N-N) inter beat intervals caused by normal heart contractions paced by sinus node depolarization. The interval between successive normal QRS complexes are measured and denoted as N-N intervals. The time domain measures include the mean normal-normal(N-N) intervals and statistical measures of the variance between NN intervals."

- Mean Heart Rate (beats per minute) – it is the average heart rate during a period of 5 minutes.
- "SDNN (ms) - it is the most representive parameter of HRV. Standard deviation of all the N-N intervals. It represents the parasympathetic activity.
- SDANN (ms) – Standard deviation of the averages of N-N intervals in all 5-minute segments of the entire recording.
- RMSSD (ms) – the square root of the mean of the sum of the squares of the differences between adjacent N-N intervals.
- NN50 count – Number of pairs of adjacent N-N intervals differing by more than 50 ms in the entire recording.
- pNN50 (%) – NN50 count divided by the total number of all N-N intervals."[41]

FREQUENCY DOMAIN PARAMETERS

Total power (TP in ms^2) – it is the total power of power spectral density in the frequency range between 0 and 0.4 Hz. The total power measures overall autonomic activity – the sympathetic activity, which is the primary contributor.

- Very Low Frequency (VLF in ms^2) – it is a band of power spectrum ranging between 0.0033 and 0.04 Hz. VLF parameter indicates the overall activity of sympathetic function.
- Low Frequency (LF in ms^2) – this power spectrum ranges between 0.04 and 0.15 Hz. This band reflects both the sympathetic and parasympathetic activity with sympathetic dominance.
- High Frequency (HF in ms^2) – this band of power spectrum range between 015 and 0.4 Hz. It reflects parasympathetic (vagal) activity.
- LF/HF Ratio - The LF/HF ratio parameter is the ratio between the power of Low frequency bands and High frequency bands. This ratio shows the overall balance between the sympathetic and the parasympathetic systems. Higher LF/HF ratio reflects the domination of the Sympathetic nervous system, whereas lower LF/HF ratio indicates the domination of the Parasympathetic nervous system.
- Normalized Low Frequency (LF nu) – it represents "the ratio between the absolute value of Low Frequency and the difference between Total power and Very Low Frequency." This measure emphasizes the changes in the sympathetic regulation. It is calculated in percentile units.
- Normalized High Frequency (HF nu) – this is "the ratio between absolute value of the High Frequency and difference between Total Power and

Very Low Frequency." This measure emphasizes the changes in the parasympathetic regulation. It is calculated in percentile units.[41]

Uses of HRV

1. It is a non- invasive method which measures both the cardiovascular and non cardiovascular autonomic functions.
2. Assesses the effectiveness of treatment and its prognosis.
3. Effectiveness of stress relaxation program like meditation ,yoga etc.
4. In sports physiology in training of athletes.
5. Morbidity predictor in post myocardial infarction, arrhythmias, etc.
6. HRV regularity gives an early warning sign in various diseases like diabetic neuropathy.[42]
7. Assessing the sympatho-vagal imbalance.

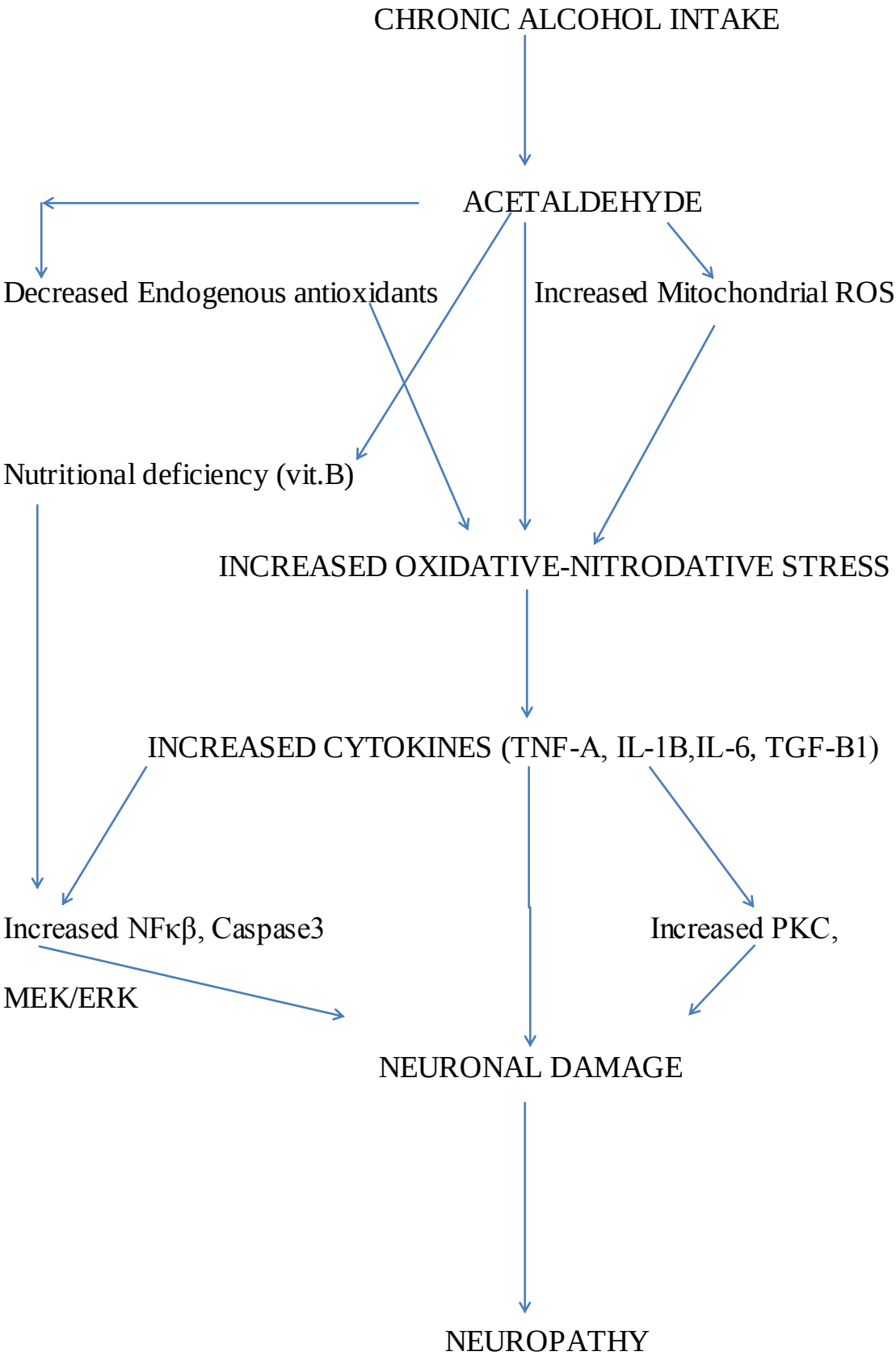
HRV IN ALCOHOLIC DEPENDENCE SUBJECTS

Chronic Alcohol dependence contributes to Alcoholic Neuropathy and a reduced HRV. The explanations for the regularity of HRV is multifaceted and they are: Chronic alcohol intake causes thiamine deficiency. This chronic deficiency leads to nerve cell degeneration, reactive gliosis, atrophy of the

cerebellum and the peripheral nerves including Autonomic nerves.[37] The damage to nerves results from long term excessive drinking.

Alcoholic neuropathy is caused by activation of microglia of the spinal cord, oxidative stress induced free radical damage, release of pro-inflammatory cytokines coupled with protein kinase activation, involvement of classical MAP kinases, involvement of the extracellular signal-regulated kinases (ERKs)[43,44], involvement of the opioidergic and hypothalamo-pituitary-adrenal system. Also Cytokines are released by alcohol metabolism in the liver. It causes autonomic dysfunction and blunting of β-adrenergic signalling contributing to reduced HRV.[45]

Cytokine-induced neural modulation also affects the brain cortex and the subcortical regions like the medullary centers.[46] Due to this, the central cardiovascular medullary center is disordered resulting in uncoupling of the cardiac pacemaker cells from their brain stem regulators. This is implicated in the regularity of the cardiac cycle.[47]


CHRONIC ALCOHOL INTAKE
ACETALDEHYDE
Decreased Endogenous antioxidants
Increased Mitochondrial ROS
Nutritional deficiency (vit.B)
INCREASED OXIDATIVE-NITRODATIVE STRESS
INCREASED CYTOKINES (TNF-A, IL-1B,IL-6, TGF-B1)
Increased NFκβ, Caspase3
Increased PKC,
MEK/ERK
NEURONAL DAMAGE
NEUROPATHY

Heart rate variability analysis is used to evaluate autonomic functions in many disorders. A recent study conducted by Thirumaran et al on alcohol dependent individuals showed thiamine deficiency to contribute to the vagal neuropathy and also the same individuals showed improvement of vagal function tests following continued abstinence.[37]

JM Ryan et al in their study reported lower indices of cardiac vagal nerve activity in Alcohol dependence subjects compared to normal volunteers in his study. He also described a positive association between chronic alcohol intake and heart rate.[48] K Murata et al in their study showed alcohol affects cardiac autonomic functions including both the sympathetic and parasympathetic activities with a predominance of reduced parasympathetic activity.[49]

R H Johnson et al in their study showed parasympathetic dysfunction, affecting vagal nerve in alcoholics, who showed depressed reflex heart rate responses.[50] Simon C Malpas et al in their study established vagal neuropathy in alcohol dependent men and suggested the reduced vagal efferent activity favours cardiac electrical instability.[11]

Robert D. NEGRU et al in their study found SDNN, LF was decreased suggesting a marked decreased sympathetic activity in alcoholics.[51]JolantaBialkowska et al in their pilot study in alcoholics showed a marked decrease in SDNN, RMSSD, pNN50 remarking reduced vagal activity.[52] Duncan et al in his study suggested chronic vagal damage as a feature of alcoholic polyneuropathy.[53]

Lambie et al described in their study that alcoholics suffer central damage to autonomic pathways resulting in disorder of parasympathetic control of heart. They explained nutritional factors to be the causative agent rather than alcohols direct toxic effect and a good nutritional supplementation to protect against the development of vagal neuropathy.[54]Behse F et al in their study proved axonal degeneration of the vagus nerve in necropsy of alcoholic neuropathy.[55]RoserMonforte et al in their study stated alcoholics exhibited reduced heart rate variability, when compared with the controls.[56]

Thayer JF et al in their study evidenced dysregulation of the Hypothalamo pituitary adrenal axis, with higher basal cortisol levels, and decreased inhibitory feedback control in alcoholics with reduced HRV.[57] Ana Isabel Penzlin et al in their study have described ethyl –toxic damage ot the vasomotor and the cardiac autonomic nerve fibers leading to autonomic imbalance. They also stated that both the sympathetic and the parasympathetic nerve fibers contributed individually to cardiac autonomic dysbalance.[58]

LIVER FUNCTION TESTS

Liver function tests are the most widely performed biochemical tests in the laboratory, which help in the diagnosis and in monitoring liver diseases. The liver function tests are broadly classified as follows:

1. " Tests to detect hepatic injury:
    a. To detect the disease, whether mild or severe; whether acute or chronic.
    b. To assess the nature of liver injury; hepatocellular or cholestasis.
2. To assess hepatic function."

Functions of the liver

- Synthetic function : the liver synthesizes the major biomolecules like plasma proteins albumin, globulins, hormonal factor, clotting factors, growth factors, bile acids, cholesterol, and phospholipids.
- Carbohydrate, lipid and amino acid metabolism.
- Bilirubin metabolism
- Detoxification functions : toxic substances entering via gut and the parenteral route are detoxified in the liver by various reactions like reduction, hydrolysis, oxidation, hydroxylation, carboxylation and demethylation and excreted.

Indications for LFTs:

1. Jaundice
2. Alcoholic liver disease
3. Suspected liver metastasis
4. Coagulation disorders
5. Therapy with statins to check hepatotoxicity
6. Any undiagnosed chronic illness
7. Master health check up

Classification of liver function tests :

" Group I – hepatic excretory function

i. Serum – bilirubin; total, conjugated, and unconjugated.

ii. Urine – bile pigments, bile salts and urobilinogen

Group II – Liver enzyme panel

i. Alanine amino transferase (ALT)

ii. Aspartate amino transferase (AST)

iii. Alkaline phosphatase (ALP)

iv. Gamma glutamyltransferase (GGT)

Group III – Synthetic function of liver

i. Total proteins

ii. Serum albumin, globulins, A/G ratio

iii. Prothrombin time

Classification based on clinical aspects

Group I : Markers of Liver dysfunction :

i. Serum bilirubin, total, conjugated

ii. Total protein, serum albumin and A/G ratio

Group II : Markers of hepatocellular injury

i. Alanine amino transferase (ALT)

ii. Aspartate amino transferase (AST)

Group III : Markers of cholestasis

i. Alkaline phosphatase (ALP)

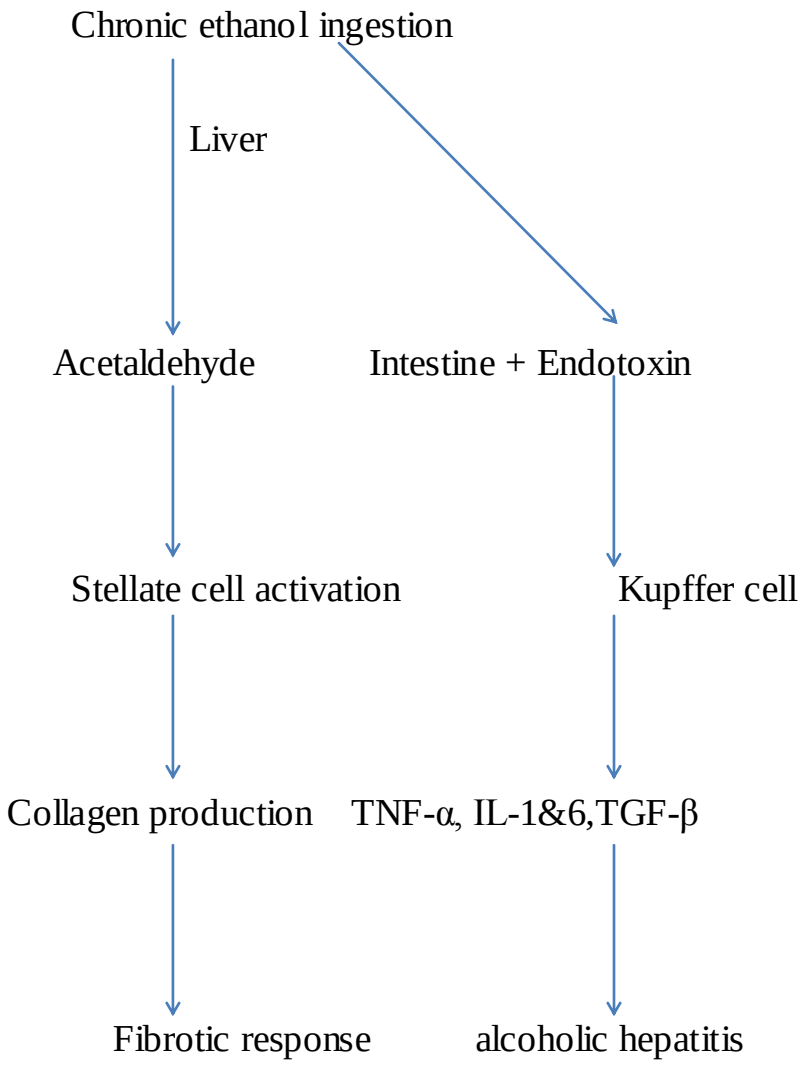
ii. Gamma glutamyltransferase (GGT)."[59]

LIVER FUNCTION TESTS IN ALCOHOL DEPENDENCE SUBJECTS :

Alcohol or ethanol is metabolized primarily in the liver, and therefore the liver is the major site of ethanol toxicity. Alcohol is a direct hepatotoxin. The metabolism of alcohol by the liver cells or hepatocytes initiates pathogenic processes leading to production of protein-aldehyde adducts, immunologic activity, lipid peroxidation, and cytokine release.

This complex interaction of alcohol with distinct hepatic cell types is crucial for alcohol induced liver injury. Stellate cell activation and collagen production are the important events occurring in hepatic fibrogenesis. This

resultant fibrosis will determine the extent of architectural derangement in the liver following chronic alcohol consumption. Also the cytokine produced is responsible for the systemic manifestations of alcoholic hepatitis.[32]



Risk factors for alcoholic liver disease:

1. Period and magnitude of alcohol ingestion: threshold intake of 40 g/d in men and 10 g/d in women.
2. Gender : females have a greater likelihood than males.

3. Genetic factors : HFE gene mutations are more common
4. Nutritional status : poor nutrition may contribute to the development of alcoholic liver disease.[60]

There are a large number of biochemical markers for the detection of excessive alcohol consumption. Typical laboratory abnormalities in alcohol dependence include modest increase in GGT, AST and ALT,also accompanied by hypercholesterolemia, hypertriglyceridemia, and hyperbilirubinemia. These serologic markers can be used in monitoring abstinence because these markers would return to normal within several weeks of cessation of drinking. An increase in values of as little as 10% indicates a resumption of heavy alcohol intake.[32]

GAMMA GLUTAMYL TRANSFERASE : Normal value :10-51 IU/L

The most widely used is Gamma-glutamyltransferase (GGT). It is a membrane bound glycoprotein enzyme, for long has been used as a sensitive marker of alcohol dependence. It roughly parallels the alcohol intake. GGT is an inducible enzyme and its threshold for positivity is 2 drinks/day. It normalizes in 2 -3 weeks of abstention.

TRANSAMINASE ENZYMES :

Normal serum ALT = 10 -35 IU/L,

Normal serum AST = 10 – 35IU/L

Serum ALT and AST are used for screening liver dysfunction in alcohol users. An increased liver transaminase (AST &ALT) is considered as an early warning sign of developing alcoholic liver disease.

ALKALINE PHOSPHATASE: Normal value : 44 – 147 IU/L

Increase in ALP enzyme activity to greater than three times the upper reference limit, is associated with higher mortality in alcoholics.

SERUM BILIRUBIN (total ,direct&indirect)

Normal values

Total bilirubin : 0.2 – 0.9 mg/dL

Direct bilirubin : 0 - 0.3 mg/dL

Hyperbilirubinemia is common is common in alcohol dependence indicating toxic damage of alcohol on liver.

TOTAL PROTEIN, ALBUMIN & GLOBULIN

Normal values

Total protein : 6 – 8.3 g/dL

Albumin: 3.4 -5.4 g/dL

Globulin: 2 – 3.5 g/dL

Reduction of liver synthesized protein concentrations are commonly present in alcoholics.Albumin is the most important protein synthesized by the liver, and it reflects the extent of functioning liver cell mass.In advanced liver injury hypoalbuminemia is common.[32]

J. B. Gogiet in their study showed increased GGT in chronic alcoholics and stated the increase in GGT was due to microsomal enzyme induction. They stated that determination of serum GGT is a useful diagnostic tool, if used judiciously and correlated correctly. Also other liver enzymes were elevated in conjunction with GGT.[61] The same findings was observed by R Teschke et al; Rosalki , S et al Wu , A et al in their study.[62,16,17]

Eri Hashimoto et al in their study titled " Consensus paper of the WFSBP task force on biological markers" have stated GGT to be an extremely sensitive and specific marker for chronic alcoholism. Also they stated that when the increase in GGT is two or more fold greater than the rise in ALP, the source of is from the liver.[63]

Subir Kumar Das et as in their study reported these biochemical markers such as GGT, AST are established markers of alcoholism. These markers are particularly useful, when a history of alcoholism is not available and also provide a prognostic information during assessment of alcoholic subjects.[26]

Ryback, R.S et al in their study demonstrated increase in the GGT levels in alcoholics and also stated it can be used in distinguishing alcoholic from non-alcoholic liver disease. Gamma glutamyl transferase is a biliary canalicular enzyme, which is induced by alcohol and raises in response to hepatocellular damage.[64]

Mitsuda Y et al in their study noticed GGT increase in parallel with the progression of alcohol induced liver disease.[65]Katkov , W.N et al in their study stated alcohol as the most common cause of elevation in Alanine amino transferase in otherwise healthy person.[66]

Hultcranz , R et al in their study reported like GGT, the amino transferases act as markers of alcohol consumption and as indicators of hepatic damage by alcohol.[67] Cohen , J.A et al in their study showed increased levels of AST and ALT.[68] Conigrave , K.M et al in their study stated elevated levels of GGT, AST and ALT in chronic alcoholics.[69]

Das BKL et al and Subirkumar et al in their respective studies described increased AST and ALT values in alcohol dependent subjects due to liver cell permeability and liver cell necrosis.[70,26] Das BKL et al reported significant increase in the liver enzymes– GGT, ALT, AST and Alkaline phosphatases in alcohol dependent subjects, and is an early indicator of liver injury.

Bilirubin is metabolized, conjugated and excreted in the liver. Alcohol is metabolized in the liver, and chronic intake causes liver damage and bilirubin elevation.[71] Das BKL et al in their study reported elevation of serum bilirubin – total and conjugated fraction is common in chronic alcoholics.[70]

Kazukirokotohet al in his study showed raised albumin levels because albumin is a potential subject of formation of adduct by alcohol metabolite acetaldehyde.[72]The same was stated by Subir Kumar et al.[26]

Das BKL et al in their study showed significantly higher albumin, AST, GGT and total protein in alcohol dependence subjects compared to normal reference value, and explained the increase may be due different drinking pattern (drinking with food or without food).[70]

3. AIM AND OBJECTIVES

AIM :

To assess the Heart rate variability and Liver function tests in Alcohol Dependent subjects.

OBJECTIVES :

The objectives of the study were

1. To assess the Heart rate variability in Alcohol dependent subjects.
2. To evaluate the Liver function parameters in Alcohol dependent subjects.

4.MATERIALS AND METHODS

The study was conducted in the Department of Physiology, Stanley Medical College, after getting approval from Institutional Ethical Committee, Stanley Medical College, Chennai.

4.1 SELECTION OF SUBJECTS :

4.1.1 SELECTION OF THE ALCOHOL DEPENDENT GROUP

Fifty five alcohol dependent individuals in the age group of 20 to 55 years were selected from the psychiatry OPD, Department of Psychiatry, Stanley Medical College and hospital, Chennai. Alcohol Dependence Subjects were chosen according to (DSM- IV) ICD-10 criteria. A diagnosis of AD is based on the presence of any three of the ICD-10 criteria within a one-year period." Subjects with a score $\geq$ 3 out of 7 were chosen as alcohol dependent group.

4.1.2 SELECTION OF THE NON-ALCOHOLIC GROUP

Fifty five age and gender matched normal volunteers attending the General Health Check Up under the Master Health Checkup Scheme Department of Community Medicine in Stanley Medical College Hospital was taken as the Non-alcoholic group for the study.

INCLUSION CRITERIA

1. Age group 20 – 55 years; both genders.

2. Patients diagnosed to have Alcohol Dependence Syndrome based on ICD-10 criteria.

EXCLUSION CRITERIA

Individuals with history suggestive of the following were excluded from the study.

1. Age <20 and >55 years.
2. H/o Hepatitis or fatty liver disease.
3. History of any other co-morbid medical illness like Diabetes, Hypertension & Cardiovascular disorder.
4. Any long term drug intake.
5. Smokers.

STUDY DESIGN : Cross Sectional – Analytical Study

PLACE OF STUDY :

Neurophysiology research laboratory, Department of Physiology, Stanley Medical College, Chennai – 1. The test was conducted between 10 am

to 1 pm. The environment of the lab was kept quiet and calm and the temperature maintained between 25 – 28°C with minimum lighting.

4.2 METHODOLOGY

4.2.1 HEART RATE VARIABILITY :

INSTRUMENT

RMS polyrite version 2.2 D hardware, which is a computerized recording system is used to acquire and analyze data. The hardware is connected to a Window based PC. The data obtained is stored in memory for analyzing in the later period. Using 2.5.2 software the RR intervals were continuously plotted. The software contains data base, filter settings and calculation tools. Following flow chart summarizes the steps in recording and processing the ECG signal to obtain HRV data.

METHODOLOGY OF HEART RATE VARIABILITY

HRV was recorded in the supine position. The study was conducted in the neurophysiology research laboratory, between 10 am to 1pm. The following precautions were followed while recording the heart rate variability.

1. The lab was kept quiet and calm and the temperature of the room was maintained between 25 to28 ‘C.

2. The subjects were made to sit comfortably, relaxed to get accustomed to the new environment.

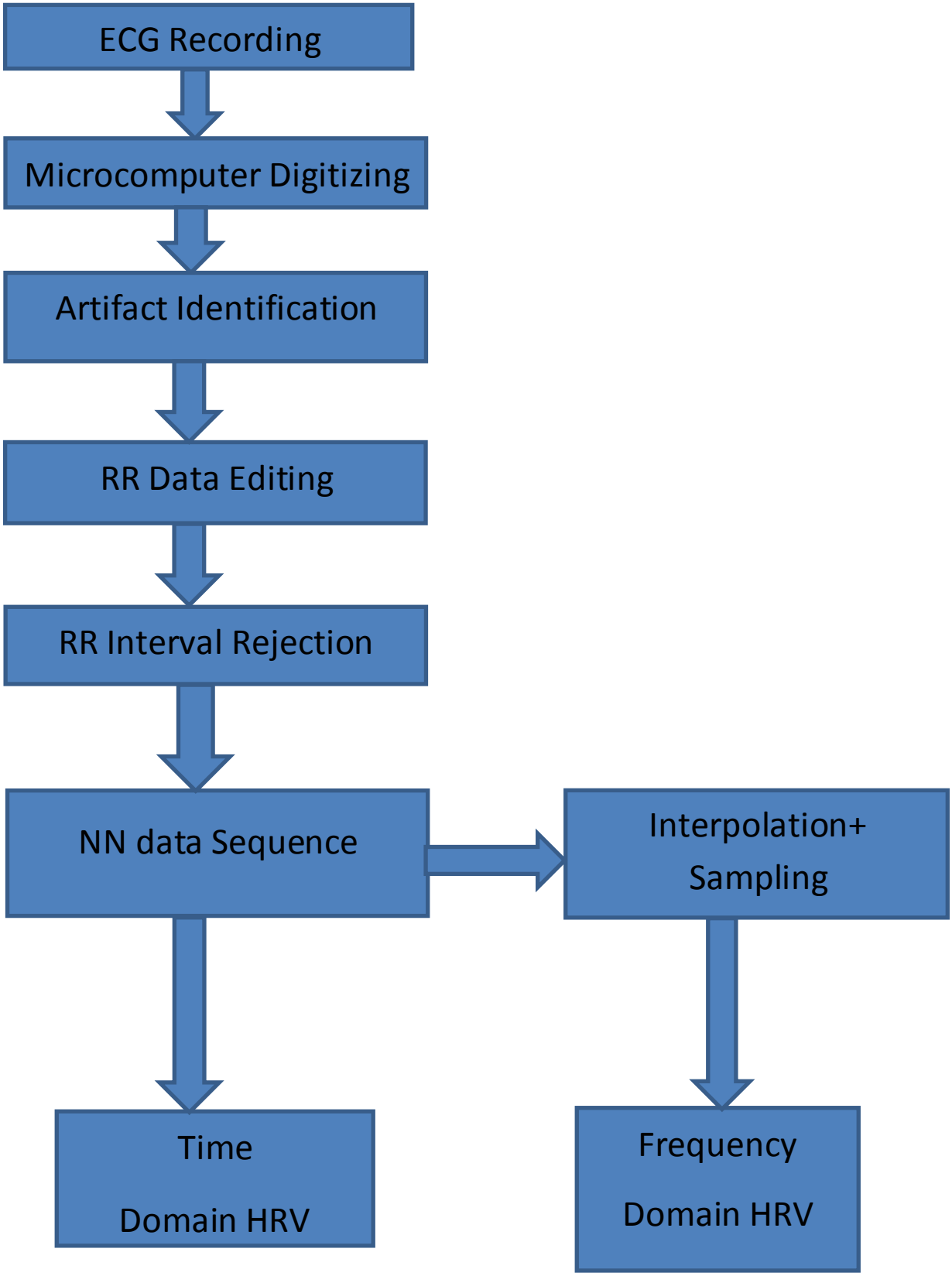
3. The test was recorded 2 hours post breakfast.

4. The subjects were asked to empty their bladder before the hrv recording.

5. Electronic gadgets like mobile phones were switched off while recording.

6. Informed written consent was obtained and a brief examination was done.

Blood pressure, heart rate and respiratory rate was recorded for both the groups.


ECG Recording
Microcomputer Digitizing
Artifact Identification
RR Data Editing
RR Interval Rejection
NN data Sequence
Interpolation+
Sampling
Time
Domain HRV
Frequency
Domain HRV

The position of electrodes for recording are as follows :

ELECTRODE	**POSITION**
Exploring Electrode	Left forearm
Exploring Electrode	Right forearm
Reference Electrode	Right leg

ECG was recorded for 10 minutes for short term analysis. The procedure was done with the subjects made to lie in supine position on a couch, awake with eyes closed. The ECG recordings with normal sinus rhythm for a period of 5 minutes was taken for analyzing the heart rate variability, excluding the artifacts.

The Task Force European Society of Cardiology guidelines was followed while recording and analyzing HRV data. Using time domain and frequency domain indices, the heart rate variability was analyzed.

To calculate the Time domain indices, each QRS complex is identified and the normal to normal (N – N) intervals (the intervals between the adjacent QRS complex) is determined.

The following indices were measured in Time domain analysis

- Mean Heart Rate
- SDNN – Standard deviation of R – R interval
- RMSSD – Root mean square of successive differences
- NN50 – Number of R – R intervals which differs by > 50 m sec from the other intervals.

The Fast Fourier Transform method (FFT) was used to obtain the spectral measures.

The following parameters were measured in frequency domain analysis

- Low frequency component (0.04 – 0.15 Hz) – marker of sympathetic activity

  High frequency component (0.16 - 0.4 Hz) - marker of parasympathetic activity
- LF/HF – reflects Sympatho-Vagal balance.

4.2.2 LIVER FUNCTION TESTS

INSTRUMENT

The liver function tests were carried out in the Central Biochemistry laboratory, Stanley Medical College. All the tests were done in the TRANSASIA BIOSYSTEM AUTOANALYSER – MODEL EM 360. It uses configurable software which allows reagents on sample position, high accuracy dispensing systems, pre-programmed racks at users option, intuitive and easy to follow software and internal quality control management.

PROCEDURE :

Liver function tests to be measured are GGT, ALT, AST, ALP, total protein, albumin, globulin, total bilirubin, direct and indirect bilirubin. They were machine calculated. The subject was made to sit comfortably. A tornique was applied in the arm above the cubitalfossa.Following aseptic precautions 2 ml of blood was withdrawn from the antecubital vein of forearm from the subject. The blood sample was then transferred to EDTA coated vacuutainersand wereappropriately labeled.Theneedles, syringe and cotton swabs were discarded appropriately.

The blood samples collected in the EDTA coated vacuutainer were placed in a centrifuger machine and centrifuged for 15 minutes at 3000 revolutions per

minute. The serum separated at the upper part of the blood column was carefully micropipetted and transferred to correspondingly labeled ependorf containers.

Quality control was checked in the Transasia Biosystem Autoanalyser, and was satisfactory. The labeled ependorf containers with the serum were positioned in racks in the machine and the results were auto calculated and depicted in the monitor. The liver function test value of both the alcohol dependent group and the non-alcoholic healthy group was obtained.

STATISTICAL ANALYSIS

Statistical analysis was done using the ‘ Unpaired t’ test. The results obtained were expressed as Mean and Standard deviation (SD). Comparison of the variables between two groups were performed using student t test and “ p ” value$<$ 0.05 was considered to be statistically significant. The data obtained were analyzed using SPSS software version 21.

5. RESULTS

5.1 DEMOGRAPHIC CHARACTERISTICS OF ALCOHOLIC AND NON-ALCOHOLIC POPULATION

The characteristics of alcoholic and non-alcoholic population are presented in Table 1 . Both the population were chosen in the age group of 20 – 55 years. The mean age of the alcohol dependence group was 37.62 ± 5.42 and non-alcoholic group was 38.04 ± 5.90. With regard to age, the distribution of subjects in the study and control group were nearly uniform.

The mean SBP in the alcoholic group was 117± 4.66 and in the non-alcoholic healthy group was 116 ± 4.94. The mean DBP in the alcoholic group was 78 ± 4.04 and in the non-alcoholic group was 77.64 ± 4.29. With regard to the SBP and DBP, the distribution of subjects in the alcoholic and non-alcoholic group showed no significant difference.

TABLE 1

Comparison of demographic parameters (mean ± SD) between Alcoholic and Non-alcoholic individuals.

Variable	Alcohol Dependence Subjects	Non – alcoholics
Age in years	38.62 ± 5.42	38.71 ± 5.49
SBP mmHg	116 ± 4.94	116 ± 4.94
DBP mmHg	78 ± 4.04	77.64 ± 4.29
BMI	22.6 ± 0.87	23.03 ± 0.72

5.2 RESTING HEART RATE

The mean heart rate in the alcoholic group was 77.82 ± 11.72 and in the non-alcoholic group was 75.27 ± 4.22. The mean heart rate is increased in alcohol dependent subjects when compared to non-alcoholic individuals and is shown in the table 2 and graph 1.

TABLE 2

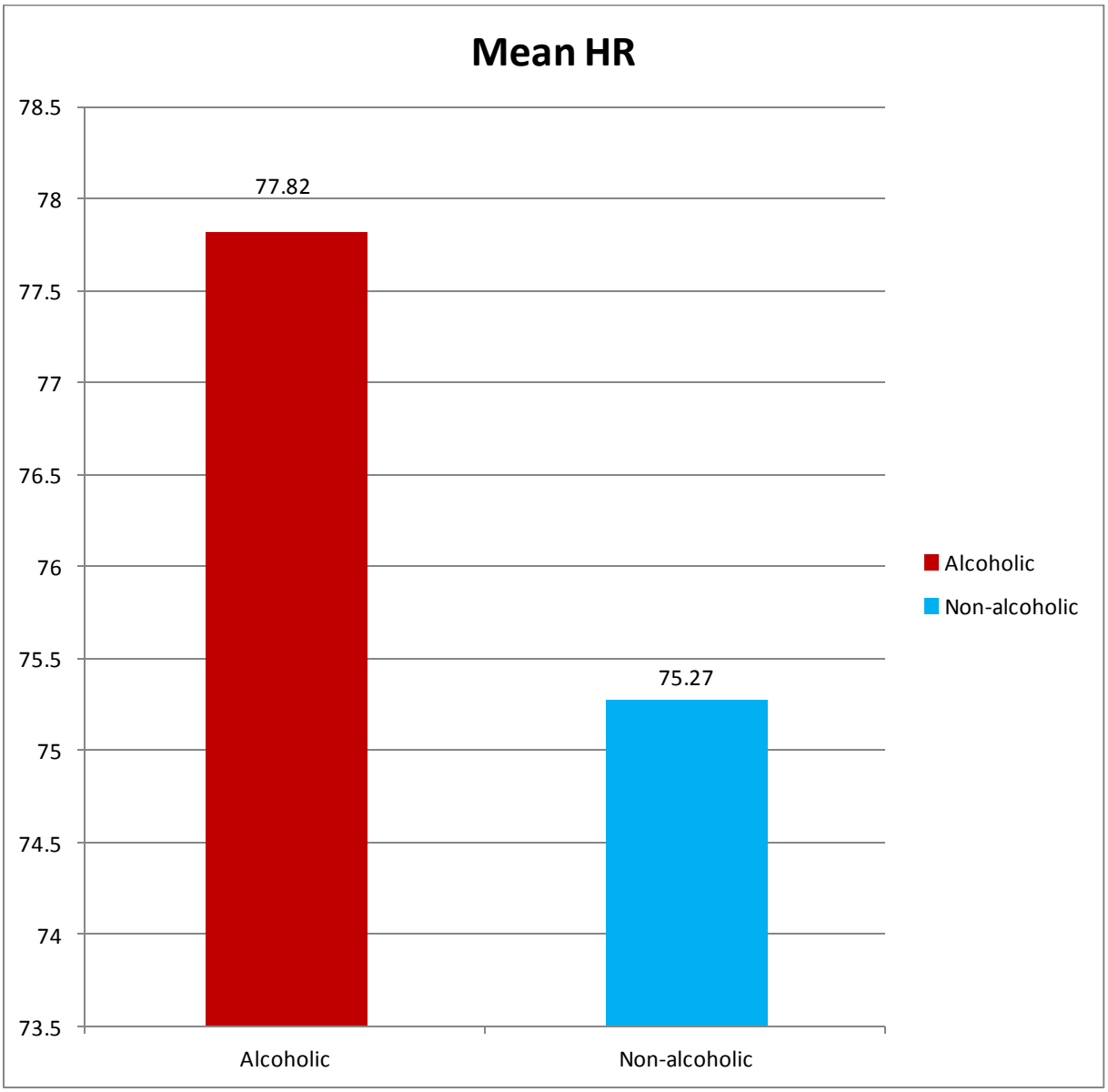
Comparison of mean values of resting heart rate between alcoholic and Non – alcoholic individuals

Variable	Alcoholics	Non-alcoholics	p value
Resting heart rate	77.82 ± 11.72	75.27 ± 4.22	$< 0.005^{**}$

$^{*}p < 0.05$ significant, $^{**}p < 0.01$ Highly significant

Graph 1

Comparison of mean values of resting heart rate between alcoholic and non-alcoholic subjects



The above graph shows the rise in mean heart rate in the alcohol dependent subjects group in comparison to the non – alcoholic healthy group.

5.3 Heart Rate Variability

Heart rate variability indices includes Time Domain parameters and Frequency domain parameters. The time domain parameters which include mean RR, SDNN, RMSSD, NN50, pNN50 are furnished in the table 3 and the same is represented in the graph 2. The Frequency domain parameters including LF, HF and LF/HF are represented in the table 4 and graphs 3 and 4.

TIME DOMAIN INDICES

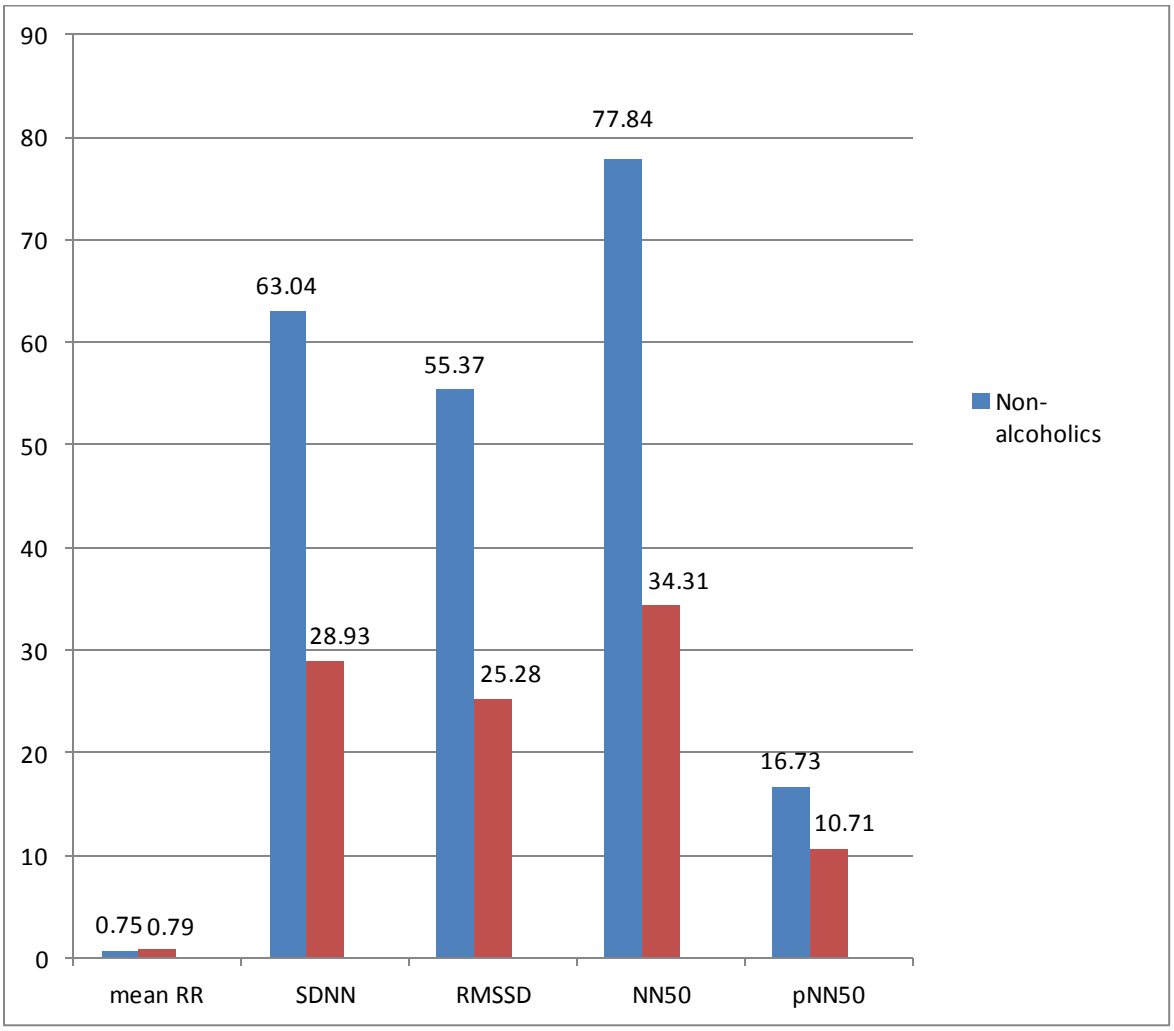
TABLE 3: Comparison of mean value of time domain indices between alcoholic dependent subjects and non-alcoholic individuals

Time domain	Alcoholics		Non-alcoholics		p – value
	Mean	SD	Mean	SD	
Mean RR	0.79	0.12	0.74	0.09	0.05*
SDNN	28.93	13.35	63.04	17.22	0.00**
RMSSD	25.28	15.97	55.37	16.10	0.00**
NN50	34.31	52.50	77.84	36.39	0.00**
pNN50	10.71	16.46	16.73	11.07	0.00**

*$p < 0.05$ significant, **$p < 0.01$ Highly significant

Graph 2

Comparison of time domain indices between alcoholic dependent subjects and non-alcoholic individuals



From the above graph 2, the time domain indices – mean RR, SDNN, RMSSD, NN50 and pNN50 were reduced in alcohol dependent subjects than in non-alcoholicsubjects.

FREQUENCY DOMAIN INDICES

Table 4

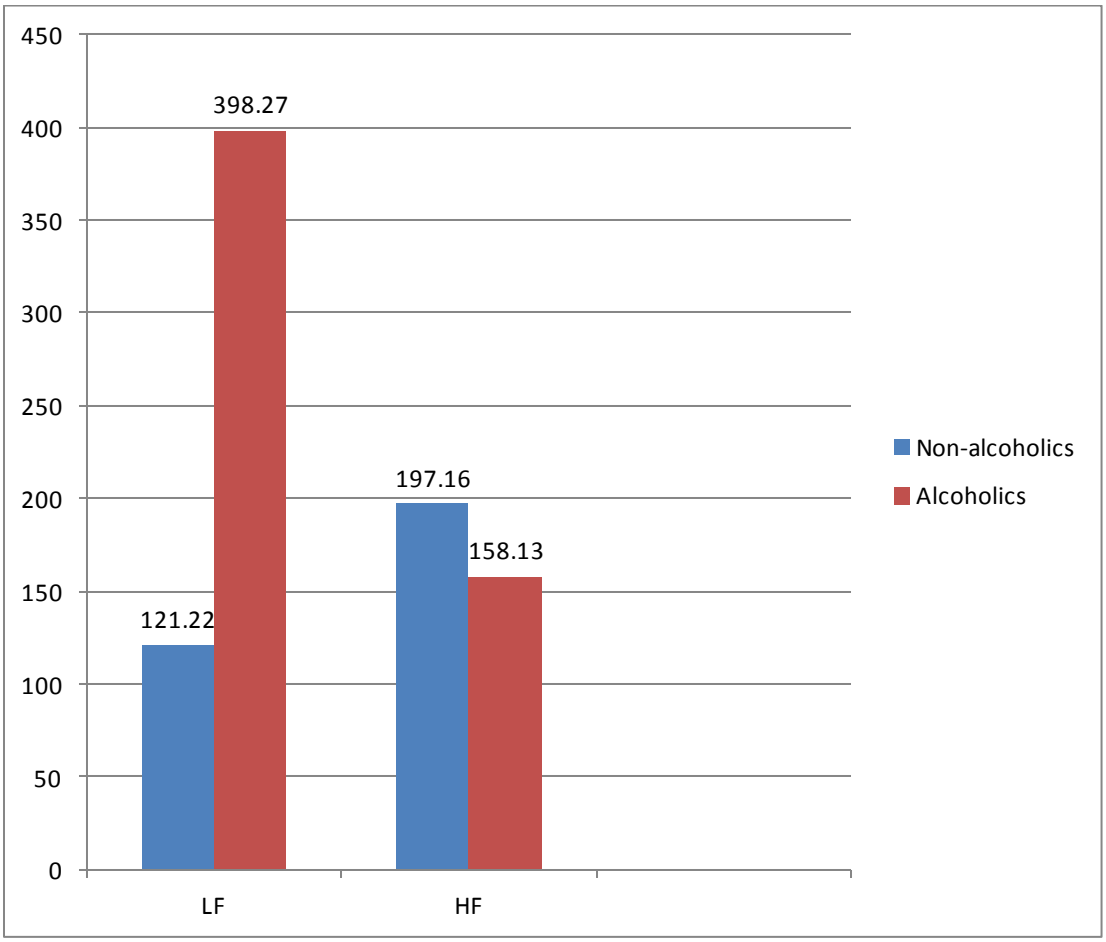
Comparison of mean values of frequency domain measures between alcohol dependent subjects and non-alcoholic individuals

Frequency domain	Non-alcoholics		Alcoholics		p - value
	Mean	SD	Mean	SD	
LF ms^2	398.27	345.15	121.22	31.27	0.00**
HF ms^2	158.13	134.99	197.16	42.29	0.00**
LF/HF	3.61	1.15	0.62	0.15	0.00**

*$p < 0.05$ significant, **$p < 0.01$ Highly significant

Graph 3

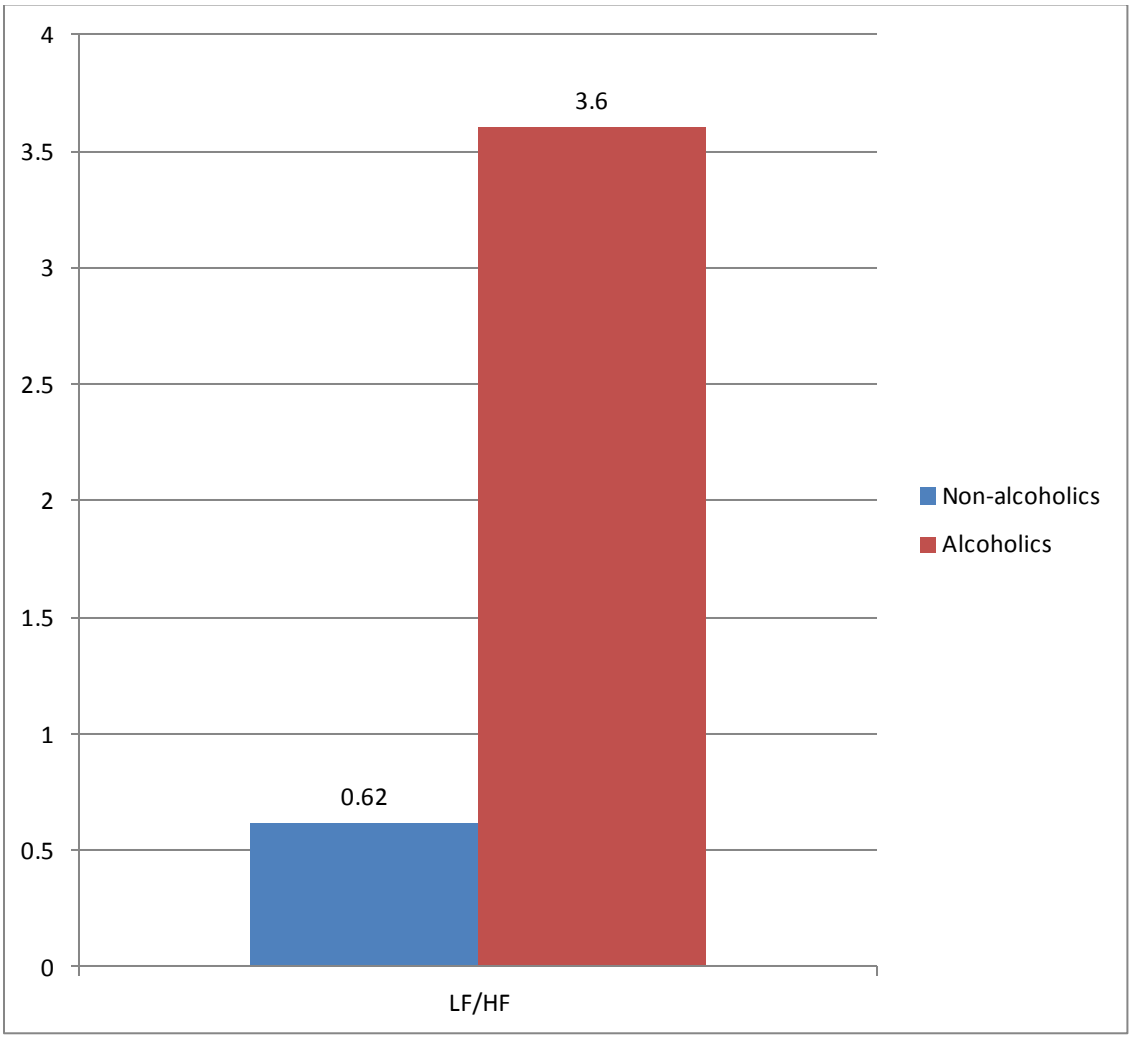
Comparison of low frequency and high frequency domain measures between alcoholic dependent subjects and non-alcoholic individuals



The above graph 3 depicts the increase in Low Frequency measure and a decrease in the High Frequency measure in alcohol dependent subjects when compared to non-alcoholic individuals.

Graph 4

Comparison of mean values of LF/HF ratio between alcohol dependent subjects and non-alcoholic individuals.



The above graph shows the increase in LF/HF measure in alcohol dependent subjects when compared to non-alcoholic individuals.

Thus the frequency domain measure LF was increased in alcoholics than in non-alcoholics, HF was decreased in alcoholics than in non-alcoholics and the ratio of LF/HF was increased in alcoholics than in non-alcoholics. The difference in the means for all the parameters werestatistically significant.

5.4 LIVER FUNCTION TEST

The mean values of the liver function parameters, comprising Gamma glutamyltransferase, SGOT, SGPT, Alkaline phosphatase,total protein, albumin, globulin, total bilirubin, direct and indirect bilirubin are compared between the alcoholic and non-alcoholic groups and are represented in the table 5, 6 & 7 and the same are represented in the graphs 5 and 6.

Table 5

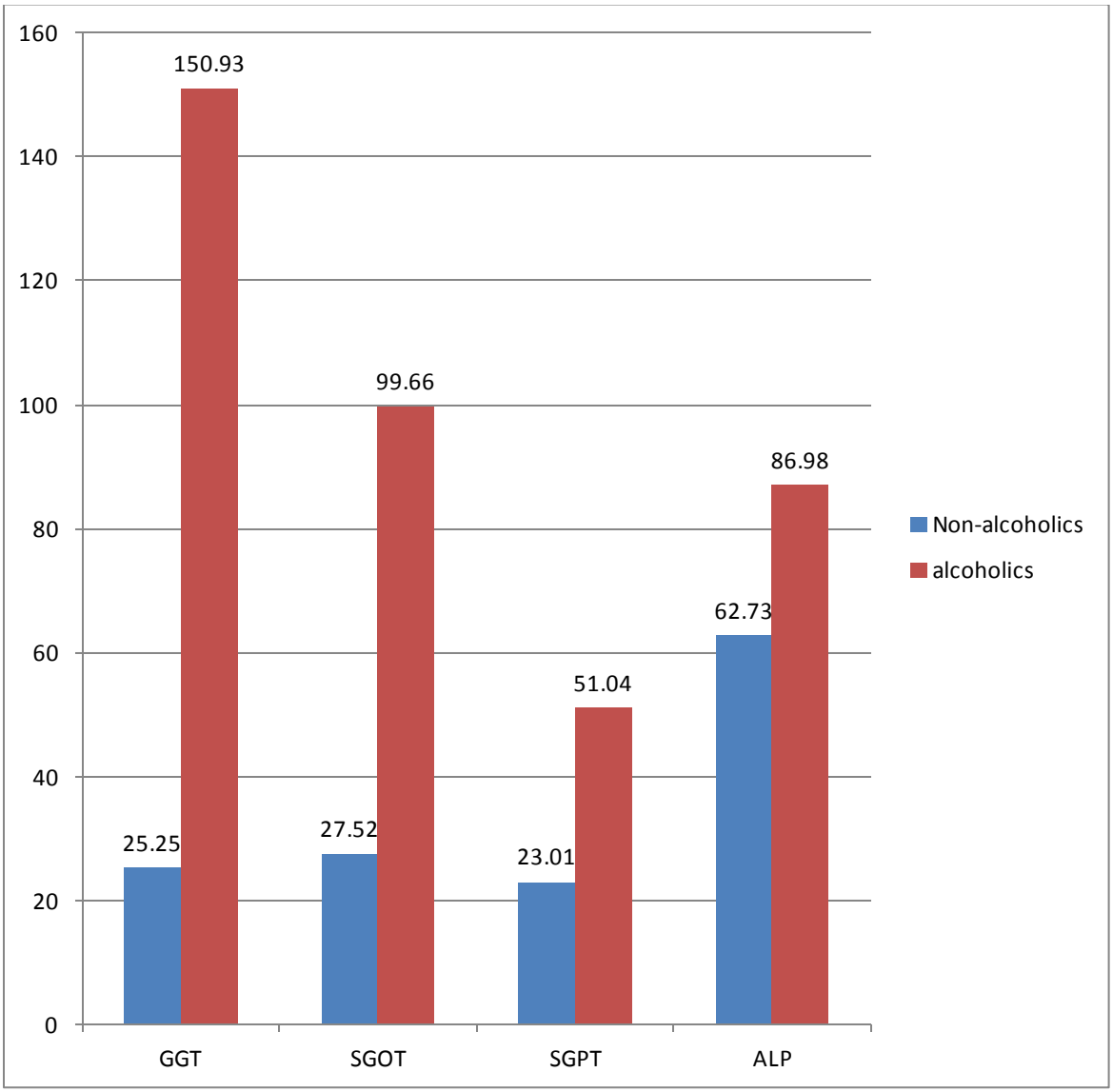
Comparison of mean value of the liver function enzymes between alcohol dependent individuals and non-alcoholic subjects

Liver function tests	Alcoholics		Non-alcoholics		p-value
	Mean	SD	Mean	SD	
GGT	150.93	191.98	25.25	9.16	0.00**
SGOT/AST	99.66	126.47	27.52	6.98	0.00**
SGPT/ALT	51.04	42.05	23.01	6.37	0.00**
ALP	86.98	32.63	62.73	16.66	0.00**

p< 0.05 significant, **p < 0.01 Highly significant

Graph 5

Comparison of mean values of the liver function enzymes between the Alcohol dependent individuals and non-alcoholic subjects



The above graph depicts the increase in liver function enzymes between alcohol dependent individuals and non-alcoholic subjects.

Table 6

Comparison of mean values of the liver function parameters between alcohol dependent subjects and non-alcoholic healthy individuals

Liver function tests	Alcoholics		Non-alcoholics		p-value
	Mean	SD	Mean	SD	
Total protein	6.70	1.05	6.62	0.32	0.6
Albumin	4.10	0.55	3.93	0.31	0.00**
Globulin	2.60	0.74	2.66	0.43	0.6

$p < 0.05$ significant, $^{**}p < 0.01$ Highly significant

Table7

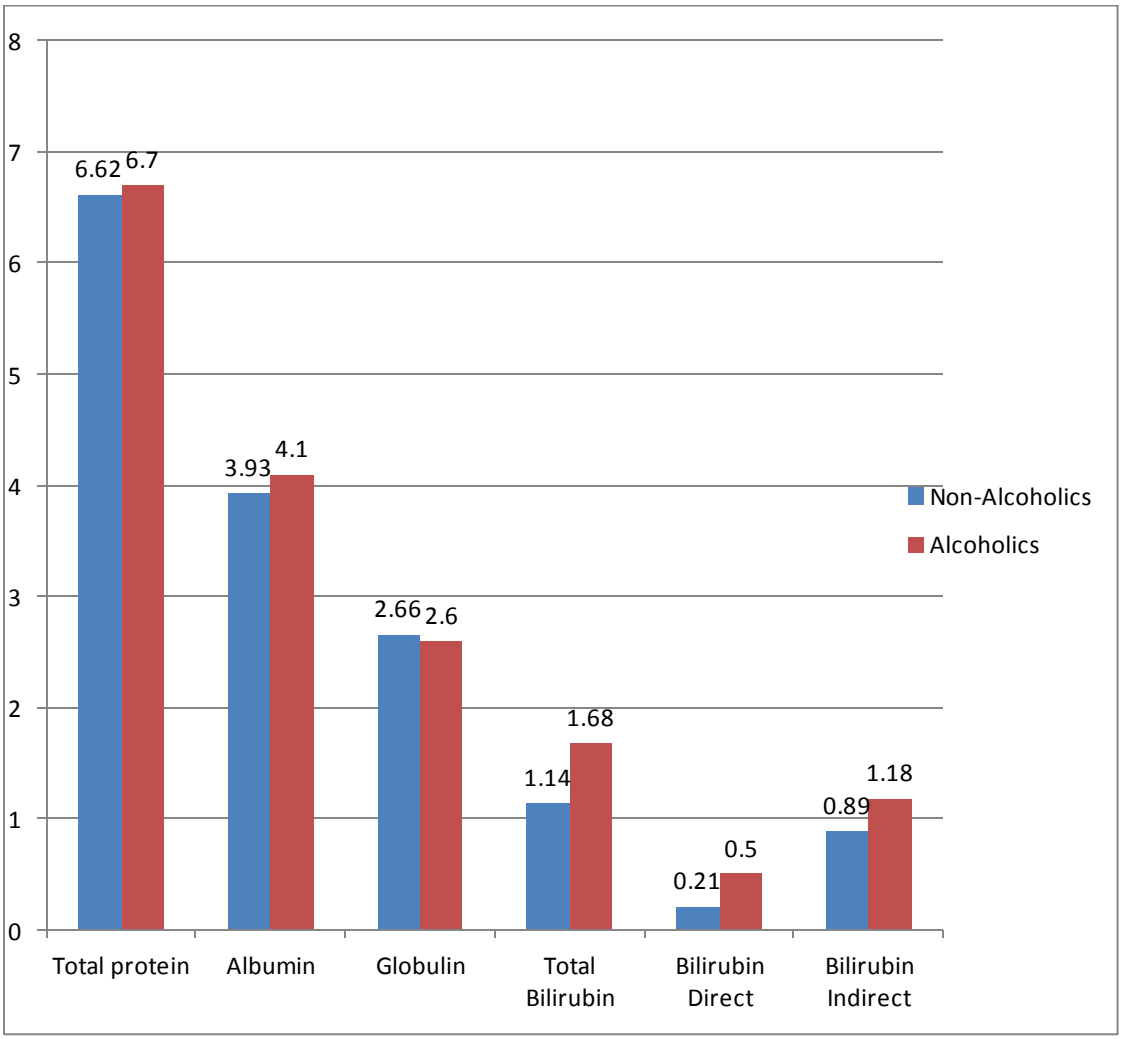
Comparison of mean values of the liver function parameters between alcohol dependent subjects and non-alcoholic healthy individuals

Liver function tests	Alcoholics		Non-alcoholics		p-value
	Mean	SD	Mean	SD	
Total Bilirubin	1.68	1.11	1.14	0.33	0.00**
Bilirubin Direct	0.50	0.51	0.21	0.08	0.00**
Bilirubin Indirect	1.18	0.70	0.89	0.35	<0.00**

p< 0.05 significant, **p < 0.01 Highly significant

Graph 6

Comparison of mean values of the liver function parameters between alcoholic dependent subjects and non-alcoholic healthy individuals.



The above graph 6 shows the increase in bilirubin in alcohol dependent subjects when compared to non-alcoholic healthy individuals.

6. DISCUSSION

The aim of our study was to assess the autonomic disturbance and estimate the liver function parameters in Alcohol Dependent individuals. Heart rate variability was employed to assess the sympathetic and parasympathetic components of the autonomic nervous system . Liver function parameters were measured using automated hematology analyzer.

6.1 CHARACTERISTICS OF ALCOHOLIC GROUP AND NON-ALCOHOLIC GROUP POPULATION

The mean age in years of Alcohol dependent individuals was 38.62 ± 5.42 which was similar to the mean age of non-alcoholic subjects 38.71 ± 5.48. Height in centimeters and weight in kilograms were measured . Body mass index (BMI) was calculated using the Quetlet formula – wt. in kg/ Ht in m^2. The mean BMI of alcoholics was 22.61 ± 0.88 and in non – alcoholic individuals was 23.03 ± 0.72 .

The mean Systolic blood pressure in alcoholics was 117 ± 4.66 and in non-alcoholic subjects was 116 ± 4.94 . The mean Diastolic blood pressure in alcoholic individuals was 77.64 ± 4.29, and the mean Diastolic blood pressure in non-alcoholic subjects was 78 ± 4.04.

Both the alcoholic and non-alcoholic groups were age, sex (male gender), BMI and blood pressure matched and showed no significant difference in their means. Hence both the alcoholic and non-alcoholic groups were comparable.

RESTING HEART RATE

The mean resting heart rate in alcoholic individuals was 77.82 ± 11.72, and in non-alcoholic individuals was 75.27 ± 4.22. The resting heart rate was found to be higher in alcoholic individuals than the non-alcoholic individuals, although the difference was not significant. This was in accordance with the findings observed by J M Ryan et al,[48] JolantaBialkowska et al[52], Ingjaldsson JT et al[73], RechlinT et al[74].

An increase in heart rate was observed in alcoholics and the reason for this positive association between heart rate and alcohol intake may be probably due to an increase in the sympathetic activity secondary to vasodilation or increased calcium entering into cardiac myocytes.[75]

6.2 HEART RATE VARIABILITY

Heart rate variability is a valuable, non – invasive tool to assess the Autonomic Nervous System function in cardiovascular diseases. Any adverse cardiovascular events are associated with changes in the resting heart rate variability. Hence, analysis of 5 minute resting heart rate variability recording

can be used to screen subjects, who are at risk of developing cardiovascular dysfunctions.

Heart rate variability was assessed using two methods namely - Time domain and Frequency domain measures. These parameters were analyzed and compared between the alcoholic and non-alcoholic individuals.

TIME DOMAIN PARAMETERS

The parameters analyzed by Time Domain Analysis are mean RR, SDNN, RMSSD, NN50 and pNN50.[41]The mean values of mean RR , SDNN , RMSSD , NN50 and pNN50 in alcoholic individuals were 0.79 ± 0.12,28.93 ± 13.35, 25 ± 15.97, 34.31 ± 52.50, 10.71 ± 16.46 respectively.

In our study mean RR, SDNN and RMSSD were reduced in alcoholics compared to non-alcoholics and significant statistical difference ($p<0.00$) was found between the alcoholic and non-alcoholic group for these three parameters, which indicates reduced parasympathetic activity on the heart. The same finding was evidenced by Thirumaran et al,[37] Simon C Malpas et al[11] and Monforte R et al[56] in their study.

Also NN50 and pNN50 values were significantly reduced ($p<0.01$), than the mean values of NN50 and pNN50 in non-alcoholic individuals. This depicts reduced vagal activity, which states reduced parasympathetic activity.

The same results were observed by Thirumaran et al,[37] Robert D.Neghru et al[51] and Urooj et al in their studies.[76]

In the Time Domain method RMSSD, NN50 and pNN50 are specific markers of vagal activity. They were reduced showing a gross reduction in vagal activity, reflecting vagal neuropathy or reduced parasympathetic activity.

R H Johnson et al did an extensive study on parasympathetic dysfunction affecting the vagus nerve in chronic heavy drinkers, who showed depressed reflex heart rate responses due to parasympathetic neuropathy. Our study supports the same indicating that alcoholic autonomic neuropathy primarily affecting the parasympathetic system than the sympathetic system.

FREQUENCY DOMAIN PARAMETERS

The parameters analyzed are LF, HF and LF/HF ratio. The meanvalues of LF and HF in ms^2 was 398.27 ± 345.15 and 158.13 ± 134.98 respectively. The LF parameter was significantly higher ($p<0.00$) in alcoholic subjects when compared to non-alcoholic individuals depicting an increase in the sympathetic activity as evidenced in the study done by D Mahesh kumar et al.[77]

The HF parameters were significantly lower ($p<0.00$) in alcoholic subjects when compared to non-alcoholic individuals indicating a decrease in the parasympathetic activity. Similar results were observed in the studies by JolantaBialkowskaet al[52] and D Mahesh kumar et al.[77]

The mean LF/HF value in alcoholics was 3.61 $\pm$ 1.15. LF/HF showed a significant increase ($p<0.00$) in alcoholic individuals compared to non-alcoholics. The analysis of LF/HF values indicate sympathetic – parasympathetic imbalance as observed by A. Fratini et al in his study.[78]

Our study, with reference to Time domain and Frequency domain measures indicate Sympatho-vagal imbalance with predominant vagal neuropathy with decreased parasympathetic activity. Our study was in favour of the studies by Thirumaran et al,[37] Simon C Malpas et al,[11] D Mahesh Kumar et al,[77] A. Fratini et al,[78] Robert D NEGRU et al,[51] Bialkowska et al.[52]

Therefore, Cardiovascular autonomic dysfunction was found in alcohol dependent subjects. The mechanism behind vagal neuropathy include the following: Cytokines released from alcohol metabolism in the liver causes autonomic dysfunction and blunting of β-adrenergic signalling contributing to reduced HRV.[45]

Cytokine-induced neural modulation affects the brain cortex and the subcortical regions like the medullary centers.[46] The central cardiovascular medullary center is disordered by cytokines, resulting in uncoupling of the cardiac pacemaker cells from their brain stem regulators. This is implicated in the reduced heart rate variability.[47]

Chronic alcohol intake causes nutritional deficiency. Chronic thiamine deficiency in alcoholics lead to nerve cells degeneration, reactive gliosis and atrophy of cerebellum and peripheral nerves including autonomic nerves. Therefore deficiency of nutritional factors is responsible for vagal neuropathy in chronic alcoholics.[37]

Chronic alcohol causes a direct dose dependent toxicity to the autonomic and peripheral nervous system.[79,56]

6.3 LIVER FUNCTION TEST

The average value of liver function test parameters in alcoholic subjects were as follows: Gamma glutamyltransferase (GGT) = 150 $\pm$ 191.98; Serum glutamic oxaloacetic transaminase (SGOT) = 99.66 $\pm$ 126.47; Serum glutamate-pyruvate transaminase (SGPT) = 51.04 $\pm$ 42.06 ; Alkaline phosphatase (ALP) = 86.98$\pm$ 32.63; Total protein = 6.70 $\pm$ 1.05; Albumin = 4.10 $\pm$ 0.55; Globulin = 2.60 $\pm$ 0.74; Total Bilirubin = 1.68 $\pm$ 1.10; Direct Bilirubin = 0.50 $\pm$ 0.51; Indirect Bilirubin = 1.18 $\pm$ 0.69.

The mean value of GGT was significantly increased ($p < 0.00$) in alcoholic subjects compared to non-alcoholic individuals. Our result was in accordance to that of Anju R et al (2017),[80] Jenny H.D.A et,[81] Conigrave KM et al,[69] Niemela et al.[82] Also the parameters SGOT , SGPT were also significantly increased ($p < 0.00$) in alcoholic subjects compared to non-alcoholic

individuals. Our results were similar to the findings of Anju R et al,[80]Conigrave et al.[69]

The mean values of Alkaline phosphatase (ALP) was significantly raised ($p< 0.00$) in alcoholic subjects compared to non-alcoholics. This finding was similar to the study results of Anju R et al (2o17),[80]Conigrave et al,[69] SubirkumarDas et al.[26]

The mean values of albumin showed a significant rise ($p < 0.00$) and our results matched with the study done by Kazukirokotoh et al.[72] The average total bilirubin levels and direct bilirubin levels showed an increase in alcoholic subjects with significance ($p<0.00$), compared to non-alcoholic individuals. The indirect bilirubin also showed an increase in alcoholics with p-value < 0.00. Our results matched with the studies done by SubirKuma Das et al[26] andAhlgren et al.[28]

Liver plays a primary role in alcohol metabolism. Increased liver enzymes in alcoholics indicates hepatic dysfunction. Elevation of liver enzymes like GGT, SGOT, SGPT,ALP are the screening tools for abnormal liver function. All the above mentioned liver enzymes are synthesized and stored inside the hepatocytes normally. Chronic alcoholism leads to hepatic cell injury. This leads to the release of liver enzymes stored within the hepatocytes into the circulation leading to elevated liver enzymes in the blood.

Gamma-glutamyltransferase is a sensitive marker of alchohol intake, liver dysfunction and oxidative stress.[83] It is a biliary canalicular enzyme induced by alcohol, and their serum levels rise in response to hepatocellular damage. Thus high levels of GGT in alcoholics indicate that they are at risk of suffering from liver disorders.

In alcohol dependent subjects, ALT and AST enzymes which are normally present in higher concentration in hepatocytes leak into the circulation when hepatocytes or their cell membranes are damaged. Elevated ALP is a marker for alcoholism and liver damage.[26]

Increased albumin was due to formation of adduct protein by acetaldehyde, which is an important product of alcohol metabolism.[26,84,85] Bilirubin is metabolized, conjugated and excreted by the liver. Hence, hepatocellular damage leads to its decreased metabolism and excretion causing hyperbilirubinaemia. Elevated bilirubin level suggests cholestasis in alcoholics and hepatic dysfunction[71,80]

LIMITATIONS OF THE STUDY

Our study was conducted with a small sample size population. Hence in the future, the study may be expanded with a larger sample size. Also, our study was done in alcohol dependent subjects based on the ICD – 10 criteria. The

study could have been done on various groups of alcohol dependent subjects based on their duration of alcohol intake. This would be done in our future studies.

Comparison of Heart Rate Variability and Liver Function Tests in Alcohol dependent subjects before and after alcohol abstinence, which will be done in our future study. Carbohydrate Deficient Transferase (CDT) is more specific along with GGT in detecting alcoholic subjects, which would be included in our future study.

FUTURE STUDY

1. Correlation between Heart Rate Variability and Liver Function Tests will be done in our future study.
2. The study will be conducted in different alcoholic variants based on their duration of alcohol intake, in our future study.
3. Comparison of Heart Rate Variability and Liver Function Tests between Alcohol Dependent subjects and after abstination of alcohol.

7. CONCLUSION

The resting heart rate variability showed a decrease in the time domain parameters (RMSSD, NN50, pNN50%) indicating reduced parasympathetic activity. In the frequency domain measures, LF was significantly increased indicating increased sympathetic activity on the heart. HF showed a significant decrease in the alcohol dependent group signifying decreased parasympathetic activity due to vagal neuropathy.

LF/HF ratio in alcoholics was significantly increased indicating Sympatho-vagal imbalance. Therefore from the results of our study on Heart rate variability, we conclude there is Sympatho-vagal imbalance with reduced parasympathetic activity in alcohol dependent individuals.

Elevated liver function enzymes and increased bilirubin levels were seen in the alcohol dependent subjects indicating the toxic effect of alcohol on liver. If intervention is done at the earliest, progression of liver cell damage to cirrhosis can be prevented.

HRV and Liver function tests can be used in alcohol dependent subjects for follow-up and to determine the prognosis. Bio-feedback of their heart rate variability and liver function tests can help in encouraging them to quit alcohol.

Thus HRV and Liver function tests can be combined and used as prognostic tool in these subjects and early intervention of them with alcohol abstinence, nutrition supplementation can prevent alcoholic cardiomyopathy and alcoholic liver disease.

8.SUMMARY

The aim of our study was to assess the heart rate variability and to evaluate the liver function tests between alcohol dependence individuals and non-alcoholic subjects. 55 alcohol dependent individuals based on DSM IV – ICD-10 criteria were chosen and 55 non-alcoholic subjects, age and gender matched were chosen for the study.

Resting heart rate variability was recorded in supine position. Blood samples were collected and serum after separation by centrifuging was used to measure the liver function tests.

Reduction in heart rate variability was seen in our study indicating Sympatho-vagal imbalance with predominant reduction in the parasympathetic activity in alcohol dependent individuals when compared to non – alcoholic subjects, indicating cardiac autonomic dysfunction with vagal neuropathy in these individuals.

The liver function tests were increased in alcoholic dependent individuals when compared to non-alcoholic individuals indicating liver cell derangement in these individuals.

Hence, heart rate variability and liver function tests can be done as a routine in all alcohol dependent individuals, to predict the autonomic dysfunction and alcohol induced liver disease at the earliest, and intervening by abstinence, life style modification and drug therapy (if necessary), can revert the health of the person back to normal and thereby improving the quality of life.

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ANNEXURES

PROFORMA

ID No :

Name :

Occupation :

Address :

Age :

Gender :

Education :

Mobile :

Guardian/Spouse name :

PR : _____/min

BP :_____mm Hg.

Height : ____cm

Weight :____kg

BMI :

Waist Circumference:_____cm .

Hip Circumference:_____cm

Waist

Hip Ratio :

Blood group :

Personal H/o :

Smoker / Non-Smoker

H/o any substance abuse

Menstrual H/o :

Family H/o : Father/Mother /Relative taking alcohol.

Alive / Dead – if dead specify reason for death.

Marital H/o : Married since _____ years.

Pedigree chart :

Diet H/o : Veg / Non – Vegetarian .

Sleep pattern : ____hrs /day.

Sleeps at _____ &wakes up at _____.

H/o sleep disturbances / Night mares :

Past H/o :

known Diabetes / Hypertension/ COPD/ TB/ Bronchial Asthma/ Cardio Vascular Disease/ Thyroid disorder.

H/o any co-morbid illness/ medical emergency : Yes / No

H/o any surgery in the past : Yes / No.

H/o RTA/ Head injury/ LOC :

H/o any previous treatment for Deaddiction: Yes / No.

- If Yes specify the reason for relapse:

H/o Drug intake for chronic illness:

General Examination :

Built:

Nourishment:

Conscious : Oriented:

Afebrile:

Anemic / Icteric/ Cyanosis/ Clubbing/

Pedal oedema/ GeneralisedLymphadenopathy :

Bowel & Bladder habits :

H/o Palpitations :

H/o Epigastricpain :

H/o Tremors :

H/o Hemoptysis/ Hemetemesis/ Malena/ Hematuria :

H/o Seizures /Hearing voices :

Mental Health : Intelligence-

Arithmetic knowledge- Memory- ,

Abstract thinking- Judgement- .

Speech :

Reason For Deaddiction treatment (at present) :

<u>Systemic Examination :</u>

CVS : RS :

P/A : CNS :

ICD – 10 Criteria to detect Alcohol Dependence : Score > 3 = Alcohol Dependence Subjects.

a. A strong desire / sense of compulsion to take the substance;
b. Difficulties in controlling substance-taking behavior in terms of its onset,termination, or levels of use;
c. A physiological withdrawal state, when substance use has ceased or been reduced, as evidenced by: the characteristic withdrawal syndrome for the substance; or use of the same substance with the intention of relieving / avoiding withdrawal symptoms;
d. Evidence of tolerance, such that increased doses of the psychoactive substances are required in order to achieve effects originally produced by lower doses;
e. Progressive neglect of alternative pleasures / interests because of psychoactive substance abuse, increased amount of time necessary to obtain or take the substance or to recover from its effects;
f. Persisting with substance use despite clear evidence of overtly harmful consequences, such as harm to the liver through excessive drinking, depressive mood states consequent to periods of heavy substance use, or drug-related impairment of cognitive functioning.

1. Known Alcoholic for past _____years.
2. Alcohol consumption started at _____years of age.
3. How did you get into alcoholism : Friends / Self/ other reasons-
4. Type of Alcohol :
5. Amount of Alcohol intake & Frequency of intake when you started
6. Amount of Alcohol intake & Frequency of intake at present :
7. Last drunk on :
8. Do you drink during work?

HRV ASSESSMENT :

(1) Time domain measures :

SDNN	
SDANN	
RMSSD	
SDSD	
pNN50	
HRV triangular	

(2) Frequency domain measures : VLF, ULF, LF, HF,LF/HF.

VLF	
ULF	
LF	
HF	
LF/HF	

.

LAB INVESTIGATIONS – LFTs :

GGT	
ALP	
AST	
ALT	
Sr. Bilirubin	
Sr. Albumin	

HRV RECORDING IN SUPINE POSITION

TRANSASIA BIOSYSTEM AUTOANALYSER

MASTER CHARTS

GROUP2	AGE	SEX	SBP	DBP	BMI	LF(ms2)	HF(MS2)	LF(n.u)	HF(n.u)	LF/HF(%)	MEAN RR(	MEAN HR(	SDNN(ms)	RMSSD	NN50	pNN50(%)	GGT (U/L)	SGOT (U/L	SGPT (U/L	ALP (U/L)	TOT.PRO	ALB.	GLOBU.(g/	TOT. BR	BR.DIR	BR.IND
C1	49	M	110	80	22.63	75	113	28.7	48.7	0.663	0.586	78	44.76	39.76	93	23.7	24.4	35.4	26.5	76	6.12	4.29	1.83	1.1	0.2	0.9
C2	45	M	110	80	23.33	107	140	32.8	48.7	0.764	0.615	76	47.34	53.48	118	19.8	34.2	33.4	39.3	67	6.23	4.12	2.11	1.21	0.2	1.01
C3	40	M	120	80	22.69	104	264	28.2	71.8	0.392	0.739	81	36.248	35.95	74	19.5	34.5	23.4	21.9	54	6.43	4.02	2.41	1.3	0.3	1
C4	41	M	120	80	23.87	153	158	51	49.2	0.965	0.743	81	23.058	20.553	4	1.1	43.4	24.5	23.5	89	6.32	3.9	2.42	1.5	0.2	1.3
C5	31	M	120	80	22.46	95	188	26.3	72.4	0.382	0.734	76	34.451	30.231	6	2	54.8	29.3	15.3	50	6.97	3.58	3.39	0.96	0.2	0.76
C6	50	M	120	70	25.1	179	190	38.5	61.7	0.942	0.707	74	68.67	52.78	102	33.1	12.8	31.2	19.8	56	6.45	3.99	2.46	1.5	0.1	1.4
C7	33	M	110	80	23.74	100	142	41.4	58.9	0.703	0.715	84	21.756	11.611	0	0	14.3	22.3	18.7	66	6.54	4.01	2.53	1.5	0.31	0.19
C8	50	M	120	80	24.25	117	186	34.3	65.7	0.629	0.715	78	58.58	62.97	118	5	23	34.4	28.5	64	7.01	4.33	2.68	1.15	0.27	0.88
C9	30	M	120	80	23.78	139	213	17.8	38.5	0.652	0.663	76	52.87	64.64	68	1.5	18.9	21.2	17.9	54	7.12	4.23	2.89	1.12	0.23	0.89
C10	38	M	110	70	23.14	112	229	48.4	51.6	0.489	0.778	66	48.91	58.05	108	20.9	43.1	46.8	17.6	108	6.86	3.88	2.98	1.16	0.5	0.66
C11	49	M	120	80	23.5	146	252	36.7	63.3	0.579	0.727	83	26.123	21.301	17	4.2	24.3	41.7	23.4	66	6.75	4.13	2.62	1.2	0.29	1.09
C12	34	M	120	80	23.33	186	204	47.5	52.5	0.906	0.744	81	28.39	28.685	37	9.4	19.2	38.3	36.4	66	6.89	4.18	2.71	0.89	0.12	0.77
C13	28	M	110	80	22.97	110	250	40.1	59.9	0.44	0.931	72	78.98	64.86	112	15.3	18.1	36.9	23.3	54	6.97	4.51	2.46	0.92	0.14	0.78
C14	35	M	120	80	23.34	92	169	36.1	63.9	0.544	0.791	76	82.76	67.78	78	22.4	27.1	28.3	21.3	45	6.56	4.23	2.33	0.89	0.18	0.71
C15	36	M	120	80	24.46	144	228	38.5	61.7	0.631	0.908	74	76.86	48.63	102	41	27.9	29.4	18.6	76	6.36	4.12	2.24	1.3	0.18	1.12
C16	40	M	120	80	22.38	94	152	34.3	65.7	0.618	0.882	78	68.98	72.98	128	8	19.2	24.3	17.7	65	6.63	4.02	2.61	1	0.21	0.79
C17	41	M	110	80	24.2	109	192	48.7	68.9	0.567	0.724	80	59.04	68.92	112	16.1	27	32	27.3	80	5.71	3.84	1.87	1.8	0.2	1.6
C18	45	M	120	80	22.45	152	305	36.8	64.7	0.498	0.823	76	69.08	71.76	61	12	13.7	22.3	15.9	55	6.88	3.6	3.28	1.32	0.43	0.89
C19	35	M	120	80	23.14	67	150	54.8	72.7	0.446	0.664	79	74.56	57.57	126	17	22.3	25.9	19.2	54	6.02	3.21	2.81	1.02	0.23	0.79
C20	45	M	110	70	22.54	110	160	28.8	45.6	0.67	0.668	72	78.46	48.87	74	12	28.3	34.6	23.2	52	6.98	3.76	3.22	1.03	0.25	0.78
C21	42	M	120	80	22.31	115	164	40.6	60.4	0.701	0.824	70	68.34	64.78	68	8.9	21.1	28.7	17.9	45	6.67	3.58	3.09	1.12	0.28	0.84
C22	40	M	110	70	23.54	115	163	41.3	58.7	0.704	0.654	82	56.418	34.667	49	15.9	22	27.8	19.3	47	6.56	3.47	3.09	1.02	0.21	0.81
C23	31	M	120	80	22.43	117	280	53.6	72.4	0.417	0.548	64	76.87	63.98	102	44.5	28.5	32.5	35	56.8	6.68	3.59	3.09	1.18	0.16	0.02
C24	35	M	120	80	23.4	125	243	46.8	62.4	0.514	0.734	74	81.98	59.09	2	14	22.6	35.4	28.5	67	7.54	4.29	3.25	1.09	0.18	0.91
C25	33	M	110	80	23.1	117	208	35.6	68.7	0.562	0.674	76	76.46	69.97	41	10	34	33.9	30.5	82	6.84	3.45	3.39	1.13	0.21	0.92
C26	50	M	120	80	22.54	184	191	49.1	50.9	0.963	0.777	77	31.486	26.364	8	2.1	32.3	16.7	33.2	88	6.78	3.42	1.36	1.42	0.31	1.11
C27	36	M	120	80	22.87	125	202	38.2	61.8	0.616	0.764	79	38.057	23.888	10	2.6	30.2	15.7	24.33	89	6.12	3.67	2.45	1.15	0.21	0.94
C28	36	M	110	70	22.9	174	272	34.8	52.7	0.639	0.865	68	59.98	68.09	98	21.6	27.2	19.8	20.1	93	6.52	3.98	2.54	1.18	0.19	0.99
C29	35	M	120	80	22.88	145	204	24.7	48.9	0.71	0.743	76	64.88	59.65	84	12.8	12.9	32.2	26.5	92	6.51	3.67	2.84	1.43	0.18	1.25
C30	36	M	110	70	22.87	97	185	29.8	46.7	0.524	0.803	78	67.84	70.08	68	18	14.6	26.5	22.5	53	6.32	3.78	2.54	1.32	0.19	1.13
C31	36	M	120	80	22.68	179	200	32.8	54.7	0.895	0.786	74	81.08	71.09	98	19.8	12.7	19.4	16.2	44	6.21	3.9	2.31	1.42	0.15	1.27
C32	35	M	110	80	22.56	89	190	29.8	49.6	0.468	0.813	76	81.88	67.98	82	18	23.8	22.8	32.8	48	6.23	4.32	1.91	1.6	0.19	1.41
C33	36	M	120	80	22.48	90	188	33.8	54.9	0.478	0.654	70	78.04	58.67	81	21.6	17.9	24.9	15.4	71	6.64	4.4	2.24	1.12	0.15	0.97
C34	36	M	110	80	22.34	102	190	32.6	54.8	0.536	0.643	68	69.98	76.06	16	32.4	11.3	31.2	13.9	82	6.72	3.64	3.08	1.32	0.16	1.16
C35	40	M	120	80	22.16	114	176	22.6	43.7	0.647	0.765	74	69.54	69.98	90	19.8	15.3	33.7	35.4	64	6.89	3.86	3.03	1.42	0.15	1.27
C36	25	M	110	80	23.14	139	213	17.8	38.5	0.652	0.663	76	52.87	64.64	68	1.5	16.4	27.3	19.2	53	6.73	3.54	3.19	1.21	0.15	1.06
C37	40	M	120	80	22.09	110	240	39.1	60.1	0.45	0.942	71	78.8	64.76	112	15.3	16.2	29.3	15.4	61.1	6.95	3.65	3.3	1.56	0.2	1.36
C38	40	M	120	80	24.09	112	226	48.5	51.7	0.492	0.779	67	49.81	58.06	107	21.9	18.2	26.2	22.3	46.9	6.89	3.98	2.91	1.22	0.16	1.06
C39	42	M	110	70	22.56	134	218	37.5	62	0.634	0.912	75	76.78	48.64	103	42	14.9	27.4	21.2	35.6	6.43	4.76	1.67	1.02	0.21	0.81
C40	36	M	120	80	23.3	98	162	36.8	64.1	0.545	0.786	76	83.78	68.87	76	24.2	22.8	21.1	17.2	42	6.6	3.98	2.62	1.09	0.23	0.86
C41	42	M	110	80	22.65	146	246	34.9	53.9	0.589	0.569	72	64.74	48.18	93	5.4	24.3	19.7	24.1	43	6.45	3.75	2.7	1.08	0.3	0.78
C42	44	M	120	80	22.45	76	114	28.9	48.9	0.667	0.589	76	45.78	39.67	92	24.6	43.2	18.8	18.2	49	6.35	3.54	2.81	1.17	0.27	0.9
C43	40	M	110	80	22.15	178	189	39.5	62.7	0.952	0.708	74	70.32	42.51	49	29.5	33.9	17.2	16.5	59	6.52	3.5	3.02	1.15	0.29	0.86
C44	45	M	120	80	22.36	72	180	34.4	66.7	0.4	0.824	72	66.75	55.45	97	36	31.6	18.8	22.8	52	6.43	3.91	2.52	1.06	0.19	0.87
C45	37	M	110	70	23.25	117	186	34.3	65.7	0.629	0.718	78	69.9	62.97	118	5	27.8	29.9	19.9	53	6.42	3.99	2.43	1.81	0.19	1.62
C46	40	M	120	80	23.97	141	212	38.5	61.7	0.631	0.908	74	78.68	48.66	102	41	28.3	25.9	15.9	59	6.87	4.18	2.69	1.23	0.2	1.03
C47	36	M	120	80	21.95	110	164	28.8	46.5	0.68	0.669	72	78.56	48.78	74	12	16.5	24.5	19.8	39	6.65	4.15	2.5	1.04	0.27	0.77
C48	40	M	110	70	24.22	94	152	33.4	65.7	0.618	0.882	79	68.98	72.98	128	8	21.9	24.6	20.1	48	6.79	4.11	2.68	1.07	0.25	0.82
C49	38	M	120	70	22.32	152	305	36.9	65.7	0.512	0.832	77	69.09	72.78	65	12	32.2	34.8	33.7	59	6.87	4.21	2.66	1.03	0.3	0.73
C50	44	M	110	80	21.95	109	192	48.9	70.1	0.578	0.743	80	59.04	69.82	112	16.2	26.2	25.6	19.2	65	6.98	4.23	2.75	1.01	0.21	0.8
C51	35	M	120	80	23.5	110	164	28.9	46.6	0.69	0.678	74	78.56	51.9	74	12	28.1	18.9	21.9	47	6.48	3.99	2.49	0.19	0.03	0.16
C52	37	M	120	80	24.17	69	150	54.8	72.7	0.446	0.665	72	75.54	58.78	126	17	31.4	30.1	28.9	65	6.46	3.89	2.57	0.21	0.14	0.07
C53	36	M	110	80	22.62	97	185	29.8	46.7	0.524	0.823	78	68.87	71.08	68	18	23.2	13.2	19.9	68	6.85	3.98	2.87	0.23	0.19	0.04
C54	42	M	120	80	23.45	179	200	38.2	57.4	0.896	0.768	74	82.08	71.08	98	19.8	36.4	24.8	29.3	98	6.92	4.01	2.91	0.32	0.18	0.14
C55	38	M	110	70	22.29	145	205	25.4	49.8	0.72	0.745	76	65.88	59.89	84	12.8	38.2	38.7	34.1	89	6.53	3.59	2.94	1.2	0.08	1.12

GROUP1	AGE(	SEX	SBP	DBP	BMI	LF(ms2)	HF(MS2)	LF(n.u)	HF(n.u)	LF/HF(%)	MEAN RR(	MEAN HR	SDNN(ms)	RMSSD	NN50	pNN50(%)	GGT (U/L)	SGOT (U/L	SGPT (U/L	ALP (U/L)	T.PRO(g/dl	ALB (g/dl)	GLOBU.(g/	TOT. BR.(m	BR. DIR.(m	BR.IND.(mg
AD1	49	M	120	80	22.63	180	107	62.8	37.2	6.04	0.771	78	16.145	9.874	0	0	185.3	37.1	25.7	116	6.93	4.3	2.63	0.77	0.03	0.74
AD2	45	M	110	80	23.33	244	82	78.7	21.3	3.684	0.801	75	13.637	11.713	2	0.5	1330.6	703.6	117.4	216	6.24	3.79	2.45	5.27	2.54	2.73
AD3	40	M	120	80	22.69	4	1	82.9	17.1	4.839	0.613	98	10.988	4.289	0	0	408.1	142.8	30.9	90	6.43	3.34	3.09	1.86	0.68	1.18
AD4	41	M	120	80	22.78	28	22	56.1	43.9	1.28	0.685	88	15.572	11.404	1	0.2	209.8	171.5	46.2	120	8.19	4.25	3.94	5.25	1.76	3.49
AD5	31	M	110	80	22.46	188	95	66.3	33.7	2.75	0.891	67	59.541	54.512	116	39.6	383	167.8	56.7	100.5	6.6	3.23	3.37	2.98	0.97	2.01
AD6	50	M	120	80	24.01	493	177	77.4	22.6	3.421	0.872	69	21.169	21.248	7	2.1	98.4	143.4	38.4	117	6.86	4.21	2.65	0.46	0.09	0.37
AD7	33	M	120	80	23.74	184	33	84.9	15.1	3.929	0.863	70	52.851	42.942	60	24	159	84.9	33.2	104	8.14	4.62	3.52	0.72	0.01	0.771
AD8	50	M	120	80	24.25	293	182	61.8	38.2	2.878	0.692	87	41.286	30.603	48	12	216	66.7	38	100	8.84	4.97	3.87	1.32	0.34	0.98
AD9	30	M	120	80	23.78	48	24	72.6	27.4	2.656	0.693	87	18.532	15.786	3	0.7	214	42.3	23.7	60	9.28	4.94	4.34	1.95	0.47	0.48
AD10	38	M	110	70	23.14	77	47	63.4	36	1.763	0.711	84	22.782	19.266	10	2.5	85.8	46.8	17.6	108	6.86	3.88	2.98	1.16	0.5	1.11
AD11	49	M	120	80	23.5	109	33	77.7	22.3	3.487	0.658	91	28.688	18.722	15	3.3	88.5	41.7	23.4	66	6.75	4.13	2.62	1.2	0.29	1.06
AD12	34	M	120	80	23.33	627	227	78.9	21.1	3.737	0.871	69	44.124	47.648	119	35.2	67.7	62.5	31.2	101	9.17	5.14	4.03	1.84	0.78	1.06
AD13	28	M	110	80	22.97	25	23	53.2	46.8	1.139	0.653	92	14.783	11.221	0	0	168.7	109.9	37.1	80	6.27	4.1	2.17	0.71	0.18	0.53
AD14	35	M	120	80	22.09	639	261	72.9	25.7	2.833	0.936	64	34.017	53.061	151	47.2	60.1	79.1	41.9	65	7.2	4.57	2.63	2.04	0.81	1.23
AD15	36	M	120	70	24.46	788	375	72.6	27.4	2.65	0.96	62	38.813	53.747	133	43.5	284.3	647.5	227	135	7.27	4.69	2.58	1.14	0.42	0.72
AD16	40	M	120	70	22.38	37	7	86.7	13.3	6.541	0.636	94	18.253	8.288	0	0	297.8	85.5	28.6	113	5.51	2.79	2.72	1.16	0.23	0.93
AD17	41	M	110	80	22.42	225	90	71.5	28.5	4.615	0.929	65	33.213	27.246	17	5.3	156	89.3	56	90	6.23	3.76	2.47	1.17	0.86	0.31
AD18	45	M	120	70	22.45	202	125	61.8	38.2	3.423	0.764	79	38.057	23.888	10	2.6	198	76.98	53	110	5.64	2.4	3.24	1.81	0.9	0.91
AD19	35	M	120	80	23.14	262	109	70.6	29.4	3.833	0.775	77	51.564	30.733	33	9.5	346.5	331	85.8	102	5.76	3.63	2.13	3.17	1.27	1.9
AD20	40	M	110	70	22.54	361	118	78.3	21.7	3.611	0.834	72	20.725	18.391	2	0.6	115.6	39.3	17.2	89	5.64	3.9	1.74	0.51	0.16	0.35
AD21	42	M	120	80	23.21	351	115	77	23	3.345	0.792	76	29.469	21.9	9	2.4	203	145.9	62.8	67	7.3	4.36	2.94	2.31	0.62	1.69
AD22	40	M	110	70	21.54	422	156	76.4	23.6	3.231	0.816	74	31.948	24.877	15	4.1	95.5	127.1	44.5	108	7.48	4.1	3.38	2.54	1.1	1.44
AD23	31	M	120	80	22.43	563	195	77.8	22.2	3.5	0.876	68	45.956	32.711	47	13.9	28.5	53.8	23.1	44	6.29	3.74	2.55	1.17	0.49	0.68
AD24	35	M	120	80	22.34	334	97	80	20	4	0.921	65	25.339	25.329	10	3.1	69.2	83.3	44.9	139	7	4.55	2.45	2.61	1.1	1.51
AD25	33	M	110	80	22.31	629	284	75.4	24.5	3.071	0.964	62	41.39	42.441	90	29.4	96.6	33.1	205.8	74	4.98	3.36	1.62	0.34	0.04	0.3
AD26	50	M	120	70	22.54	797	261	80	21.8	3.667	1.052	57	36.04	27.895	23	7.7	32.3	16.7	33.2	41	4.78	3.42	1.36	1.42	0.48	0.94
AD27	36	M	120	80	22.42	2030	743	75.2	24.8	3.038	0.629	95	25.821	15.504	3	1.1	162.3	117.2	51.1	55	5.56	4.04	1.52	2.45	1.11	1.34
AD28	36	M	110	70	21.19	467	155	79.2	20.8	3.813	0.866	69	16.981	17.448	0	0	33.6	59.5	32.8	49	6.22	4.04	2.18	1.4	0.44	0.96
AD29	35	M	120	80	22.44	856	351	77.2	22.8	3.385	1.053	57	40.034	62.307	155	55.2	211.4	47.1	40.6	47	4.02	2.71	1.3	0.38	0.07	0.31
AD30	36	M	110	80	23.78	867	301	77.8	22.2	3.5	1.009	59	32.324	34.79	36	12.3	29	45	48.8	87	6.15	4.37	1.78	0.69	0.19	0.5
AD31	36	M	120	70	23.63	75	82	68.8	31.2	2.208	0.712	84	28.978	48.032	189	45.8	12.4	32.9	30.6	61	6.59	4.18	2.41	0.96	0.21	0.75
AD32	35	M	120	80	23.36	211	103	67.3	32.7	6.541	0.636	94	18.253	8.288	0	0	15.3	37.4	25	71	6.42	4.1	2.32	0.87	0.03	0.84
AD33	36	M	120	80	22.48	162	40	82.9	17.1	4.852	0.724	83	16.852	8.464	2	0.5	25.2	40.8	26.3	100	6.36	4.16	2.2	0.63	0.11	0.52
AD34	36	M	120	80	22.34	702	243	76.8	23.3	3.316	0.795	75	31.254	20.154	4	1.3	20.3	38.7	27.3	88	6.81	4.42	2.39	0.88	0.26	0.62
AD35	40	M	120	80	22.16	694	241	76.8	23.2	3.316	0.795	75	31.207	20.128	4	1.2	23.9	49.5	42.6	75	6.16	4.1	2.06	1.47	0.57	0.9
AD36	25	M	110	80	21.43	246	72	80.9	18.3	4.417	0.616	97	12.092	7.95	0	0	178.2	46.8	28	112	5.93	4.05	1.88	1.85	0.69	1.16
AD37	40	M	120	80	21.09	966	315	78	22	3.556	0.798	75	55.921	45.972	83	35.6	44.7	67.9	68.9	52	6.58	4.27	2.31	2.29	0.51	1.78
AD38	40	M	120	80	24.09	620	173	80.9	19.4	4.167	0.636	94	8.388	8.449	1	0.3	29	37.1	27	83	6.26	4.34	1.9	0.98	0.32	0.66
AD39	42	M	120	70	22.56	167	116	59.2	40.8	4.167	0.636	94	8.388	8.449	1	0.3	28.3	35	20.8	61	6.52	4.3	2.22	2.49	0.89	1.6
AD40	36	M	120	80	23.13	550	242	74	26.9	2.75	0.823	73	50.844	44.621	85	24.6	21.4	46.2	30.6	46	6.65	4.36	2.29	0.61	0.07	0.54
AD41	42	M	110	80	22.65	801	317	77.3	22.7	3.4	0.97	62	29.577	28.43	17	5.6	38.5	48.9	6.2	96	7.32	4.45	2.87	1.95	0.31	1.64
AD42	44	M	120	80	22.45	157	58	75.5	24.5	3.086	0.742	81	18.138	12.097	0	0	15.7	5	25.7	95	6.23	4.03	2.2	1.71	0.16	1.55
AD43	40	M	110	80	22.15	794	446	65.3	33.7	1.939	0.846	71	49.219	59.677	149	47.8	22.8	30.2	26	42	6.12	4.07	2.05	1.04	0.2	0.84
AD44	45	M	120	80	21.34	641	411	64	36	1.775	0.859	70	50.046	61.05	166	49.4	28.3	47.4	37.4	48	7.59	4.65	2.94	2.61	0.71	1.9
AD45	37	M	120	80	23.25	391	124	80.2	19.8	4.056	0.815	74	18.278	13.133	0	0	17.2	32.6	21.5	74	6.24	4.24	2	2.47	0.96	1.51
AD46	40	M	120	80	23.28	478	142	79.5	20.5	3.889	0.847	71	21.635	17.473	2	0.6	129.8	154	126.2	32	7.13	4.65	2.48	0.77	0.01	0.76
AD47	36	M	120	80	21.95	246	72	80.9	18.3	4.417	0.616	97	12.092	7.95	0	0	327.4	231.3	137.9	151	9.72	4.53	5.19	5.07	1.65	3.42
AD48	40	M	120	70	20.88	148	124	72.6	27.4	2.656	0.693	87	18.532	15.789	3	0.7	40.2	32.9	26	90	6.38	3.9	2.48	0.67	0.13	0.54
AD49	38	M	120	70	21.23	162	40	82.9	17.1	4.852	0.724	83	16.852	8.464	2	0.5	163.2	78.9	82.4	96	6.49	4.02	2.47	0.81	0.01	0.8
AD50	44	M	110	80	21.95	137	70	86.7	13.3	6.541	0.636	94	18.253	8.288	0	0	198.3	74	69.2	94	6.44	3.81	2.63	1.81	0.17	1.64
AD51	35	M	120	80	23.15	136	100	82.3	17.7	4.656	0.622	96	20.504	16.835	1	1.4	189.2	112	55.5	67	6.01	4.01	2	1.92	0.08	1.84
AD52	37	M	120	80	22.13	334	97	80	20	4.01	0.921	65	25.339	25.329	10	3.1	176.5	79.3	54.7	73.4	8.1	5.3	2.8	1.76	0.12	1.64
AD53	36	M	120	80	21.62	422	156	76.4	23.6	3.231	0.816	74	31.948	24.877	15	4.1	132.5	39.9	35.4	58	6.89	4.12	2.77	1.89	0.18	1.71
AD54	42	M	110	70	20.45	229	78	77.4	22.6	3.423	0.764	79	38.057	23.888	10	2.6	154	95.3	78.8	102	7.34	4.23	3.11	1.62	0.09	1.53
AD55	38	M	120	80	22.29	106	39	78.4	21.8	3.602	0.735	82	40.322	26.679	28	5.9	234.5	89	80.5	123.2	6.56	3.99	2.57	1.5	0.02	1.48